Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	65	sphingosine adj kinase$	US-PGPUB; USPAT	OR	OFF	2004/02/26 11:19
L2	29	1 same (gene$1 or sequence$1)	US-PGPUB; USPAT	OR	OFF	2004/02/26 11:20

```
  * * * * * * * * * * * * * * STN  Columbus  * * * * * * * * * * * * * * *

FILE 'HOME' ENTERED AT 11:37:38 ON 26 FEB 2004

=> fil .bec
COST IN U.S. DOLLARS                                    SINCE FILE      TOTAL
                                                           ENTRY    SESSION
FULL ESTIMATED COST                                         0.21       0.21

FILES 'MEDLINE, SCISEARCH, LIFESCI, BIOTECHDS, BIOSIS, EMBASE, HCAPLUS, NTIS,
       ESBIOBASE, BIOTECHNO, WPIDS' ENTERED AT 11:37:57 ON 26 FEB 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

11 FILES IN THE FILE LIST

=> s sphingosine kinase#
FILE 'MEDLINE'
          3870 SPHINGOSINE
        204599 KINASE#
L1         233 SPHINGOSINE KINASE#
                 (SPHINGOSINE(W)KINASE#)

FILE 'SCISEARCH'
          3447 SPHINGOSINE
        232141 KINASE#
L2         275 SPHINGOSINE KINASE#
                 (SPHINGOSINE(W)KINASE#)

FILE 'LIFESCI'
           760 "SPHINGOSINE"
         66216 KINASE#
L3          75 SPHINGOSINE KINASE#
                 ("SPHINGOSINE"(W)KINASE#)

FILE 'BIOTECHDS'
            70 SPHINGOSINE
          7369 KINASE#
L4          27 SPHINGOSINE KINASE#
                 (SPHINGOSINE(W)KINASE#)

FILE 'BIOSIS'
          3853 SPHINGOSINE
        261104 KINASE#
L5         282 SPHINGOSINE KINASE#
                 (SPHINGOSINE(W)KINASE#)

FILE 'EMBASE'
          3143 "SPHINGOSINE"
        180354 KINASE#
L6         230 SPHINGOSINE KINASE#
                 ("SPHINGOSINE"(W)KINASE#)

FILE 'HCAPLUS'
          4567 SPHINGOSINE
        217107 KINASE#
L7         291 SPHINGOSINE KINASE#
                 (SPHINGOSINE(W)KINASE#)

FILE 'NTIS'
            27 SPHINGOSINE
          1475 KINASE#
L8           3 SPHINGOSINE KINASE#
                 (SPHINGOSINE(W)KINASE#)
```

```
FILE 'ESBIOBASE'
          1576 SPHINGOSINE
         96315 KINASE#
L9         198 SPHINGOSINE KINASE#
                 (SPHINGOSINE(W)KINASE#)

FILE 'BIOTECHNO'
          1429 SPHINGOSINE
         92256 KINASE#
L10        142 SPHINGOSINE KINASE#
                 (SPHINGOSINE(W)KINASE#)

FILE 'WPIDS'
           328 SPHINGOSINE
          8696 KINASE#
L11         34 SPHINGOSINE KINASE#
                 (SPHINGOSINE(W)KINASE#)

TOTAL FOR ALL FILES
L12       1790 SPHINGOSINE KINASE#

=> s l12(10a)gene/q
FILE 'MEDLINE'
L13          9 L1 (10A)GENE/Q

FILE 'SCISEARCH'
L14         14 L2 (10A)GENE/Q

FILE 'LIFESCI'
L15          7 L3 (10A)GENE/Q

FILE 'BIOTECHDS'
L16         11 L4 (10A)GENE/Q

FILE 'BIOSIS'
L17          8 L5 (10A)GENE/Q

FILE 'EMBASE'
L18         13 L6 (10A)GENE/Q

FILE 'HCAPLUS'
L19         51 L7 (10A)GENE/Q

FILE 'NTIS'
L20          0 L8 (10A)GENE/Q

FILE 'ESBIOBASE'
L21         12 L9 (10A)GENE/Q

FILE 'BIOTECHNO'
L22         10 L10(10A)GENE/Q

FILE 'WPIDS'
L23         10 L11(10A)GENE/Q

TOTAL FOR ALL FILES
L24        145 L12(10A) GENE/Q

=> s l24 not 2000-2004/py
FILE 'MEDLINE'
       2147689 2000-2004/PY
L25          1 L13 NOT 2000-2004/PY

FILE 'SCISEARCH'
```

```
       4083489 2000-2004/PY
L26          2 L14 NOT 2000-2004/PY

FILE 'LIFESCI'
        412849 2000-2004/PY
L27          1 L15 NOT 2000-2004/PY

FILE 'BIOTECHDS'
         79921 2000-2004/PY
L28          0 L16 NOT 2000-2004/PY

FILE 'BIOSIS'
       2215398 2000-2004/PY
L29          2 L17 NOT 2000-2004/PY

FILE 'EMBASE'
       1858806 2000-2004/PY
L30          1 L18 NOT 2000-2004/PY

FILE 'HCAPLUS'
       4030530 2000-2004/PY
L31          1 L19 NOT 2000-2004/PY

FILE 'NTIS'
         65222 2000-2004/PY
L32          0 L20 NOT 2000-2004/PY

FILE 'ESBIOBASE'
       1175148 2000-2004/PY
L33          1 L21 NOT 2000-2004/PY

FILE 'BIOTECHNO'
        491187 2000-2004/PY
L34          1 L22 NOT 2000-2004/PY

FILE 'WPIDS'
       3652597 2000-2004/PY
L35          0 L23 NOT 2000-2004/PY

TOTAL FOR ALL FILES
L36         10 L24 NOT 2000-2004/PY

=> dup rem l36
PROCESSING COMPLETED FOR L36
L37          3 DUP REM L36 (7 DUPLICATES REMOVED)

=> d tot

L37  ANSWER 1 OF 3  SCISEARCH  COPYRIGHT 2004 THOMSON ISI on STN
TI   Cytosine methylation pattern in the sphingosine kinase
     gene locus relates to the expression of the splicing variants.
SO   MOLECULAR BIOLOGY OF THE CELL, (NOV 1999) Vol. 10, Supp. [S], pp. 574-574.
     Publisher: AMER SOC CELL BIOLOGY, PUBL OFFICE, 9650 ROCKVILLE PIKE,
     BETHESDA, MD 20814.
     ISSN: 1059-1524.
AU   Imamura T (Reprint); Ohgane J; Tanaka S; Shiota K
AN   1999:980021  SCISEARCH

L37  ANSWER 2 OF 3  BIOSIS  COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
TI   Cytosine methylation pattern in the sphingosine kinase
     gene locus relates to the expression of the splicing variants.
SO   Molecular Biology of the Cell, (Nov., 1999) Vol. 10, No. SUPPL., pp. 99a.
     print.
     Meeting Info.: 39th Annual Meeting of the American Society for Cell
```

Biology. Washington, D.C., USA. December 11-15, 1999. The American Society for Cell Biology.
CODEN: MBCEEV. ISSN: 1059-1524.
AU Imamura, Takuya [Reprint author]; Ohgane, Jun [Reprint author]; Tanaka, Satoshi [Reprint author]; Shiota, Kunio [Reprint author]
AN 2000:38394 BIOSIS

L37 ANSWER 3 OF 3 MEDLINE on STN DUPLICATE 1
TI Molecular cloning and functional characterization of murine sphingosine kinase.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Sep 11) 273 (37) 23722-8.
Journal code: 2985121R. ISSN: 0021-9258.
AU Kohama T; Olivera A; Edsall L; Nagiec M M; Dickson R; Spiegel S
AN 1998395082 MEDLINE

```
=> fil .becpat
COST IN U.S. DOLLARS                                SINCE FILE      TOTAL
                                                       ENTRY    SESSION
FULL ESTIMATED COST                                    26.83      27.04

FILES 'BIOTECHDS, HCAPLUS, WPIDS' ENTERED AT 11:42:49 ON 26 FEB 2004
ALL COPYRIGHTS AND RESTRICTIONS APPLY.  SEE HELP USAGETERMS FOR DETAILS.

3 FILES IN THE FILE LIST

=> s l24 and wo/pc and pry<=1999 range=2000,
FILE 'BIOTECHDS'
         28264 WO/PC
         17979 PRY<=1999
                 (PRY<=1999)
L38          1 L16 AND WO/PC AND PRY<=1999

FILE 'HCAPLUS'
        189258 WO/PC
        362947 PRY<=1999
L39          5 L19 AND WO/PC AND PRY<=1999

FILE 'WPIDS'
        408471 WO/PC
       1379845 PRY<=1999
                 (PRY<=1999)
L40          2 L23 AND WO/PC AND PRY<=1999

TOTAL FOR ALL FILES
L41          8 L24 AND WO/PC AND PRY<=1999

=> dup rem l41
PROCESSING COMPLETED FOR L41
L42          5 DUP REM L41 (3 DUPLICATES REMOVED)

=> d tot

L42  ANSWER 1 OF 5  HCAPLUS  COPYRIGHT 2004 ACS on STN
TI   Human and Drosophila sphingosine-1-phosphate lyase and/or sphingosine
     kinase, and their use for the modulation of sphingolipid metabolism and/or
     signaling in cancer diagnosis and therapy
SO   PCT Int. Appl., 134 pp.
     CODEN: PIXXD2
IN   Saba, Julie D.; Fyrst, Henrik
AN   2003:591305  HCAPLUS
DN   139:145823
     PATENT NO.        KIND  DATE          APPLICATION NO.  DATE
     ---------------   ----  --------      ---------------  --------
```

PI WO 2003062390 A2 20030731 WO 2003-US1739 20030117 <--
W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
US 2003175939 A1 20030918 US 2002-53510 20020117 <--
US 2003219782 A1 20031127 US 2003-348052 20030117

L42 ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2004 ACS on STN
TI Cloning of cDNAs for sphingosine-1-phosphate lyases and sphingosine kinases from human and Drosophila, and their use for modulation of sphingolipid metabolism and/or signaling in cancer diagnosis and therapy
SO U.S. Pat. Appl. Publ., 49 pp., Cont.-in-part of U.S.Ser. No.356,643.
CODEN: USXXCO
IN Saba, Julie D.; Fyrst, Henrik
AN 2003:737283 HCAPLUS
DN 139:257275

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI US 2003175939	A1	20030918	US 2002-53510	20020117	<--
US 6423527	B1	20020723	US 1997-939309	19970929	
US 6569666	B1	20030527	US 1999-356643	19990719	<--
US 2003059922	A1	20030327	US 2002-286175	20021030	<--
WO 2003062390	A2	20030731	WO 2003-US1739	20030117	<--

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

L42 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1
TI Human **sphingosine kinase gene**
SO PCT Int. Appl., 91 pp.
CODEN: PIXXD2
IN Allen, Janet; Gosink, Mark; Melendez, Alirio J.; Takacs, Laszlo
AN 2001:320122 HCAPLUS
DN 134:337616

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE	
PI WO 2001031029	A2	20010503	WO 2000-EP9498	20001027	<--
WO 2001031029	A3	20020228			

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

BR 2000015138 A 20020716 BR 2000-15138 20001027 <--

EP 1228221 A2 20020807 EP 2000-971299 20001027 <--
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL
JP 2003512072 T2 20030402 JP 2001-533164 20001027 <--

L42 ANSWER 4 OF 5 BIOTECHDS COPYRIGHT 2004 THOMSON DERWENT/ISI on STN
TI Novel sphingosine-kinase protein and nucleic acid molecules for diagnosis, prophylaxis and treatment of rheumatoid arthritis, asthma, atherosclerosis, inflammation, meningitis, multiple sclerosis and septic shock;
involving vector plasmid pGEM4Z-mediated gene transfer for expression in Escherichia coli
AU Pitson S M; Wattenberg B W; D'Andrea R J; Gamble J R; Vadas M A
AN 2001-03254 BIOTECHDS
PI **WO** 2000070028 23 Nov 2000

L42 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3
TI Cloning and cDNA **sequences** of human **sphingosine kinase** isoforms SKA, SKB and SKC and therapeutic uses
SO PCT Int. Appl., 81 pp.
CODEN: PIXXD2
IN Munroe, Donald; Gupta, Ashwani; Falzone, Germaine R.
AN 2000:628278 HCAPLUS
DN 133:218538

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000052173	A2	20000908	WO 2000-CA223	20000302 <--
WO 2000052173	A3	20010215		

W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

=> log y

COST IN U.S. DOLLARS	SINCE FILE ENTRY	TOTAL SESSION
FULL ESTIMATED COST	28.21	55.25

STN INTERNATIONAL LOGOFF AT 11:48:16 ON 26 FEB 2004

PGPUB-DOCUMENT-NUMBER: 20040014635

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040014635 A1

TITLE: Sphingosine kinase and uses thereof

PUBLICATION-DATE: January 22, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Vadas, Mathew	Stirling		AU	
Gamble, Jennifer	Stirling		AU	
Xia, Pu	Magill		AU	
Wang, Lijen	Magill		AU	

APPL-NO: 10/ 275686

DATE FILED: June 25, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
AU	PQ7447	2000AU-PQ7447	May 11, 2000

PCT-DATA:
APPL-NO: PCT/AU01/00539
DATE-FILED: May 11, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 514/1

ABSTRACT:

The present invention relates generally to a method of modulating the growth of cells and, more particularly, to a method of down-regulating the growth of neoplastic cells. The present invention is useful, inter alia, in the therapeutic and/or prophylactic treatment of cancers such as, but not limited to, solid cancers such as cancers of the colon, stomach, lung, brain, bone, oesophagus, pancreas, breast, ovary or uterus.

---------- KWIC ---------

Detail Description Paragraph - DETX (30):
[0055] Said proteinaceous molecule may be derived from natural, recombinant or synthetic sources including fusion proteins or following, for example, natural product screening. Said non-proteinaceous molecule may be derived from natural sources, such as for example natural product screening or may be chemically synthesised. The present invention contemplates chemical analogs of said sphingosine kinase capable of acting as agonists or antagonists of said sphingosine kinase. Chemical agonists may not necessarily be derived from said sphingosine kinase but may share certain conformational similarities. Alternatively, chemical agonists may be specifically designed to mimic certain

physiochemical properties of said sphingosine kinase. Antagonists may be any compound capable of blocking, inhibiting or otherwise preventing said sphingosine kinase from carrying out its normal biological functions (for example N,N-dimethylsphingosine or DL-threo-dihydrosphingosine). Antagonists include monoclonal antibodies specific for said sphingosine kinase, or parts of said sphingosine kinase, and antisense nucleic acids which prevent transcription or translation of genes or mRNA in the subject cells. Modulation of expression may also be achieved utilising antigens, RNA, ribosomes, DNAzymes, RNA aptamers, antibodies or molecules suitable for use in co-suppression.

Detail Description Paragraph - DETX (39):

[0064] "Derivatives" include fragments, parts, portions, mutants, variants and mimetics from natural, synthetic or recombinant sources including fusion proteins. Parts or fragments include, for example, active regions of sphingosine kinase. Derivatives may be derived from insertion, deletion or substitution of amino acids. Amino acid insertional derivatives include amino and/or carboxylic terminal fusions as well as intrasequence insertions of single or multiple amino acids. Insertional amino acid sequence variants are those in which one or more amino acid residues are introduced into a predetermined site in the protein although random insertion is also possible with suitable screening of the resulting product. Deletional variants are characterized by the removal of one or more amino acids from the sequence. Substitutional amino acid variants are those in which at least one residue in the sequence has been removed and a different residue inserted in its place. An example of substitutional amino acid variants are conservative amino acid substitutions. Conservative amino acid substitutions typically include substitutions within the following groups: glycine and alanine; valine, isoleucine and leucine; aspartic acid and glutamic acid; asparagine and glutamine; serine and threonine; lysine and arginine; and phenylalanine and tyrosine. Additions to amino acid sequences include fusions with other peptides, polypeptides or proteins.

Detail Description Paragraph - DETX (86):

[0111] Screening for the modulatory agents hereinbefore defined can be achieved by any one of several suitable methods known to those of skill in the art including, but in no way limited to, contacting a cell comprising the sphingosine kinase gene or functional equivalent or derivative thereof with an agent and screening for the modulation of sphingosine kinase protein production or functional activity, modulation of the expression of a nucleic acid molecule encoding sphingosine kinase or modulation of the activity or expression of a downstream sphingosine kinase cellular target. Detecting such modulation can be achieved utilising techniques such as Western blotting, electrophoretic mobility shift assays and/or the readout of reporters of sphingosine kinase activity.

Detail Description Paragraph - DETX (87):

[0112] It should be understood that the sphingosine kinase gene or functional equivalent or derivative thereof may be naturally occurring in the cell which is the subject of testing or it may have been transfected into a host cell for the purpose of testing. Further, the naturally occurring or transfected gene may be constitutively expressed--thereby providing a model useful for, inter alia, screening for agents which down regulate sphingosine kinase activity, at either the nucleic acid or expression product levels, or the gene may require activation--thereby providing a model useful for, inter alia, screening for agents which up regulate sphingosine kinase expression.

Further, to the extent that a sphingosine kinase nucleic acid molecule is transfected into a cell, that molecule may comprise the entire sphingosine kinase gene or it may merely comprise a portion of the gene such as the portion which regulates expression of the sphingosine kinase product. For example, the sphingosine kinase promoter region may be transfected into the cell which is the subject of testing. In this regard, where only the promoter is utilised, detecting modulation of the activity of the promoter can be achieved, for example, by ligating the promoter to a reporter gene. For example, the promoter may be ligated to luciferase or a CAT reporter, the modulation of expression of which gene can be detected via modulation of fluorescence intensity or CAT reporter activity, respectively.

Detail Description Paragraph - DETX (90):

[0115] As detailed earlier, reference to "sphingosine kinase" should be understood as a reference to either the sphingosine kinase expression product or to a nucleic acid molecule encoding sphingosine kinase. It should also be understood as a reference to a portion or fragment of the sphingosine kinase molecule such as the regulatory region of the sphingosine kinase nucleic acid molecule. Alternatively, the molecule may comprise the binding/active portion of the expression product. In this regard, the sphingosine kinase nucleic acid molecule and/or expression product is expressed in a cell. The cell may be a host cell which has been transfected with the sphingosine kinase nucleic acid molecule or it may be a cell which naturally contains the sphingosine kinase gene.

PGPUB-DOCUMENT-NUMBER: 20040005563

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20040005563 A1

TITLE: Methods of diagnosis of ovarian cancer, compositions and methods of screening for modulators of ovarian cancer

PUBLICATION-DATE: January 8, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Mack, David H.	Menlo Park	CA	US	
Gish, Kurt C.	San Francisco	CA	US	

APPL-NO: 10/ 173999

DATE FILED: June 17, 2002

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60372246 20020412 US

non-provisional-of-provisional 60350666 20011113 US

non-provisional-of-provisional 60315287 20010827 US

non-provisional-of-provisional 60299234 20010618 US

US-CL-CURRENT: 435/6, 435/183 , 435/320.1 , 435/366 , 435/69.1 , 435/7.23 , 536/23.2

ABSTRACT:

Described herein are genes whose expression are up-regulated or down-regulated in ovarian cancer. Related methods and compositions that can be used for diagnosis and treatment of ovarian cancer are disclosed. Also described herein are methods that can be used to identify modulators of ovarian cancer.

CROSS-REFERENCES TO RELATED APPLICATIONS.

[0001] This application is related to U.S. Ser. No. U.S. Ser. No. 60/299,234, filed Jun. 18, 2001; U.S. Ser. No. 60/315,287, filed Aug. 27, 2001; U.S. Ser. No. 60/350,666, filed Nov. 13, 2001; and U.S. Ser. No. 60/372,246, filed Apr. 12, 2002, each of which is incorporated herein by reference for all purposes.

---------- KWIC ---------

Detail Description Table CWU - DETL (86):
36TABLE 14A Pkey ExAccn UniGene ID Unigene Title Pred Protein Dom R1 421296 NM_002666 Hs 103263 perilipin perilipin, SS 32.5 453028 AB006532 Hs 31442 RecQ protein-like 4 DEAD, helicase_C, Fork_head 27.6 422310 AA316622 Hs 98370 cytochrome P450, subfamily IIS SS, TM, pkinase, fn3, ig 26.5 437897 AA770561 Hs 146170 hypothetical protein FLJ22969 SS, TM, zf-DHHC 26.3 446374

AA329256 Hs 24756 ESTs, Moderately similar to al 22.6 441021 AW578716 Hs. 7644 H1 histone family, member 2 22.3 409518 BE384836 Hs 3454 KIAA1821 protein SS 21.3 413436 AF238083 Hs 68061 sphingosine kinase 1 DAGKc 21.2 424420 BE614743 Hs 146688 prostaglandin E synthase MAPEG, SS, TM, MAPEG 20.7 422645 L40027 Hs. 118890 glycogen synthase kinase 3 alp pkinase, SS, Ets 20.7 422098 H03117 Hs 111497 similar to mouse neuronal prot TM 20.2 429556 AW139399 Hs 98988 ESTs SS, pkinase, PMP22_Claudin 20.1 436485 X59135 Hs. 156110 immunoglobulin kappa constant SS, ig, SS 19.9 423652 AF052122 Hs 130712 Homo sapiens clone 23929 mRNA ABC1, SS, PID, PID 19.8 431773 BE409442 Hs 268557 pleckstrin homology-like domai PH, SS, LIM, Troponin 19.4 422179 AF091619 Hs 112667 dynein, axonemal, intermediate WD40, SS 19.3 420839 AI792682 Hs 282960 hypothetical protein MGC10870 SS, DS, UPF0139, Glyco_hydro 18.5 441356 BE384361 Hs. 182885 ESTs, Weakly similar to JC5024 SS, TM, ank 18.5 424659 AW891298 Hs 331601 Homo sapiens, Similar to cyste SS, Fork_head 18.4 439924 AI985897 Hs. 125293 ESTs SS 18.1 458814 AI498957 Hs 170861 ESTs, Weakly similar to Z195_H SS, TM, ldl_recept_a, ldl_re 17.5 451643 M64437 Hs 234799 breakpoint cluster region RhoGEF, RhoGAP, PH, C2 17.2 439108 AW163034 Hs 6467 synaptogynn 3 Synaptogynn, SS, TM, PDZ, WD 16.9 432945 AL043683 hypothetical protein FLJ10803 SS 16.8 410418 D31382 Hs 63325 transmembrane protease, serine SS, TM, ldl_recept_a, trypsi 16.8 438424 AI912498 Hs 25895 hypothetical protein FLJ14996 SS, TM 16.7 409435 AI810721 Hs 95424 ESTs SS 16.4 418969 W33191 Hs 28907 hypothetical protein FLJ20258 SH3, SH3 16.2 421612 AF161254 Hs 106196 8D6 antigen ldl_recept_a, SS, TM 16.0 456177 NM_012391 Hs 79414 prostate epithelium-specific E Ets, SAM_PNT 15.7 414837 U24266 Hs 77448 aldehyde dehydrogenase 4 famil aldedh 15.6 432631 H08379 Hs 165563 hypothetical protein DKFZp434N TM, DnaJ, UBA, ArfGap, homeob 15.5 454017 AW023617 Hs. 347130 hypothetical protein FLJ22709 SS, TM, myosin_head, RA, DAG.sub.-- 15.5 401278 Target Exon Band_41 15.4 444804 AI084452 Hs 22158 hypothetical protein FLJ21988 SS 15.4 410259 AK000337 Hs 61485 hypothetical protein GFO_IDH_MocA, GFO_IDH_MocA 15.4 406620 M81105 Hs 146550 myosin, heavy polypeptide 9, n myosin_head, Myosin_fail, I 15.1 423081 AF262992 Hs 123159 sperm associated antigen 4 TM 14.9 421495 AI583067 Hs 149152 ESTs, Weakly similar to RHOP M 14.7 416893 AA455588 Hs. 62406 hypothetical protein FLJ22573 SS, rrm, SS 14.7 413244 AW955951 Hs 159265 kruppel-related zinc finger pr SS, TM, BTB, pep_M12B_propep 14.6 406901 M14624 gb: Human 4-beta-galactosyltran 14.6 416006 AA324251 Hs. 78950 branched chain keto acid dehyd E1_dehydrog 14.6 436186 BE390717 Hs 5074 similar to S pombe dim1 DIM1, SS 14.5 455557 AW995839 gb QV4-BN0044-110200-108-h07 B Metallophos 14.4 434518 H56995 Hs 37372 homo sapiens DNA binding pepti SS 14.2 421489 AI922821 Hs. 32433 ESTs SS, PI-PLC-X, PI-PLC-Y, C2 14.1 444441 AW613841 Hs 301394 hypothetical protein MGC3101 14.0 435017 AA336522 Hs 12854 angiotensin II, type I recepto 14.0 446572 AV659151 Hs 282961 ESTs 13.9 434068 AA977935 Hs 127274 ESTs SS 13.7 432481 AW451645 Hs. 151504 homo sapiens cDNA FLJ11973 fis SS, Collagen, COLF1, TSPN 13.7 447304 Z98883 Hs 18079 phosphatidylinositol glycan, c SS, Peptidase_C2 13.6 421182 AA284855 Hs 104480 ESTs SS, Topoisomerase_I, TopoiS 13.3 407767 W15398 Hs 38628 hypothetical protein SS, zf-CCCH 13.3 456642 AW451623 Hs 109752 putative c-Myc-responsive 13.3 437457 AA757900 Hs. 270823 ESTs, Weakly similar to S65657 SQS_PSY 13.2 430178 AW449612 Hs 152475 ESTs SS 13.1 430399 AI916284 Hs. 199671 ESTs Sec7, PH 12.9 436725 BE045223 Hs. 136912 hypothetical protein MGC10796 12.9 410219 T98226 Hs 171952 occludin SS, TM, Occludin, BIR 12.7 442620 C00138 Hs 8535 Homo sapiens mRNA for KIAA1668 SS, RNA_pol_K 12.7 439233 AA831893 Hs 292767 hypothetical protein FLJ23109 zf-C3HC4, TM, Sulfate_trans 12.7 425018 BE245277 Hs 154196 E4F transcription factor 1 zf-C2H2, LIM, SS, Exo_endo_p 12.6 423801 NM_015071 Hs. 132942 GTPase regulator associated wi RhoGAP, SH3, PH 12.6 417826 T85105 Hs. 15471 ESTs SS, cadherin, Cadherin_C_te 12.6 409261 BE315042 Hs 19210 hypothetical protein MGC11308 12.6 420568 F09247 Hs 247735 protocadherin alpha 10 cadherin, SS, TM, cadherin 12.6 411570 BE144584 Hs 314341 ESTs 12.5 430397 AI924533 Hs 105607 bicarbonate transporter relate

HCO3_cotransp, SS, TM 12.5 423767 H18283 Hs 132753 F-box only protein 2 F-box, SS, F-box, HORMA 12.4 441805 AA285136 Hs 301914 neuronal specific transcriptio LIM, SS, LIM 12.3 402365 Target Exon SS, SS, TM, ig 12.2 414371 AI905865 thymosin, beta 4, X chromosome Thymosin 12.2 446780 R31107 gb yh61g01 s1 Soares placenta 12.1 428782 X12830 Hs. 193400 interleukin 6 receptor SS, TM, fn3, ig, SS, TM 12.1 427695 R88483 Hs 172862 intron of Bicaudal D homolog 1 12.1 400460 C11002253* gi.vertline.129091.vertline.sp.vertline.P23267 SS, TM, SCAN, zf-C2H2, KRAB 12.0 407341 AA918886 Hs 204918 ESTs, Weakly similar to ALU8_H SS, TM 12.0 424049 AB014524 Hs 138380 KIAA0624 protein SS 11.9 422872 BE326786 Hs 187646 ESTs TM 11.9 450800 BE395161 Hs 1390 proteasome (prosome, macropain SS 11.8 428648 AF052728 Hs. 188021 potassium voltage-gated channe cNMP_binding 11.7 432329 NM_002962 Hs 2960 S100 calcium-binding protein A S_100, efhand, SS, efhand, S.sub.-- 11.7 417061 AI675944 Hs 188691 Homo sapiens cDNA FLJ12033 fis CTF_NFI 11.6 451195 U10492 Hs 438 mesenchyme homeo box 1 homeobox, SS 11.5 417595 AA424317 Hs. 6259 KIAA1698 protein SS, TM, Glyco_hydro_31, Glyc 11.5 426500 NM_014638 Hs 170156 KIAA0450 gene product SS 11.4 433124 U51712 Hs 13775 hypothetical protein SMAP31 11.4 444001 AI095087 Hs 152299 ESTs, Moderately similar to S6 11.4 419298 AA853479 Hs 89890 pyruvate carboxylase CPSase_L_chain, PYC_OADA, H 11.4 428593 AW207440 Hs. 185973 degenerative spermatocyte (hom SS 11.3 411408 U76666 Hs 69949 calcium channel, voltage-depen ion_trans, SS, TM 11.2 404438 Target Exon 11.2 427448 BE246449 Hs 2157 Wiskott-Aldrich syndrome (ecze WH1, PBD, WH2, SS 11.2 406230 Target Exon 11.2 432125 AW972667 Hs. 183006 Homo sapiens cDNA FLJ12300 fis Band_41, ERM 11.2 408832 AW085690 Hs 63428 ESTs, Weakly similar to Z195_H 11.1 400206 Eos Control SS, SS, Glyco_tranf_43, COLF 11.1 450503 R35917 Hs 301338 hypothetical protein FLJ12587 SS 11.0 407605 W03512 Hs 6479 hypothetical protein MGC13272 SS, Sema, pkinase, TIG, PSI, e 11.0 432143 AL040183 Hs 123484 Homo sapiens, clone IMAGE 4178 SS, TM, cys_rich_FGFR 10.9 446839 BE091926 Hs 16244 mitotic spindle coiled-coil re Troponin, SS, glycolytic_en 10.8 443559 AI076765 Hs 269899 ESTs, Moderately similar to AL SS, TM, BIR, UQ_com 10.8 411298 AW835858 gb PM0-LT0017-031299-001-h07 L 10.8 409557 BE182896 Hs 211193 ESTs 10.8 435158 AW663317 Hs 65588 DAZ associated protein 1 rrm, SS, rrm 10.8 444410 BE387360 Hs 33719 ESTs, Moderately similar to S6 SS 10.6 428948 BE514362 FK506-binding protein 3 (25 kD) FKBP, PIP5K 10.6 424707 BE061914 Hs. 10844 Homo sapiens cDNA FLJ14476 fis SS, SS, TM, Sema 10.6 416819 U77735 Hs. 80205 pim-2 oncogene pkinase, SS, TM, OTU, K_tetra 10.5 419341 N71463 Hs 118888 ESTs, Weakly similar to ALU1_H SS, TM, UPF0016 10.5 444359 AI697160 Hs 143594 ESTs, Weakly similar to HS4L_H 10.5 404333 C7001735* gi.vertline.7768636.vertline.dbj.v- ertline.BAA95 vwd 10.5 401210 C12000519 gi.vertline.7710046.vertl- ine.ref.vertline.NP_05 10.5 457941 AI004525 Hs 14587 ESTs, Weakly similar to AF1518 SS, TM, SS, TM 10.4 401594 NM_024817. Homo sapiens hypothe 10.3 441790 AW294909 Hs. 132208 ESTs 10.3 444008 BE544855 Hs 236572 ESTs, Weakly similar to SFR4_H SS, SS, SAC3_GANP 10.3 438185 Y19188 Hs 320461 ESTs SS 10.2 432031 AF039196 Hs. 272367 hairless protein (putative sin jmjC 10.2 410471 T88872 gb yd31a12 s1 Soares fetal liv 10.1 433573 AF234887 Hs 57652 cadherin, EGF LAG seven-pass G SS, TM, 7tm_2, EGF, cadherin, 10.1 417371 N74613 Hs 269149 ESTs 10.0 428167 AA770021 Hs 16332 ESTs SS, ig, fn3 10.0 419563 AA526235 Hs. 193162 Homo sapiens cDNA FLJ11983 fis 10.0 412674 X04106 Hs 74451 calpain 4, small subunit (30 K) efhand, SS, CAP_GLY 10.0 425863 U43604 Hs. 159901 Human unidentified mRNA, parti 9.9 442739 NM_007274 Hs 8679 cytosolic acyl coenzyme A thio Acyl-CoA_hydro, SS, TM 9.9 429469 AA64590 Hs 27 glycine dehydrogenase (decarbo GDC-P, GDC-P 9.9 420029 BE258876 Hs 94446 polyamine-modulated factor 1 aldo_ket_red, SS, TM, gla 9.8 445625 BE246743 hypothetical protein FLJ22635 SS, TM 9.8 435339 AI358300 ESTs SS, ras 9.8 407235 D20569 Hs 169407 SAC2 (suppressor of actin muta SS, TM, Ribosomal_S13, Galac 9.8 428758 AA433988 Hs 98502 CA125 antigen, mucin 16 SS 9.8 401349 inositol polyphosphate-1-phosp 9.7 437915 AI637993 Hs 202312 Homo sapiens clone N11 NTera2D 9.7

Detail Description Table CWU - DETL (108):
42TABLE 16A Pkey ExAccn UniGene ID Unigene Title Pred. Protein Dom. R1
407223 H96850 gb yw03b12 s1 Soares melanocyt 58.9 430281 AI878842 Hs 237924
CGI-69 protein mito_carr 46.7 410418 D31382 Hs 63325 transmembrane protease,
serine Idl_recept_a, trypsin 41.0 431773 BE409442 Hs.268557 pleckstrin
homology-like domai PH 37.1 438424 AI912498 Hs.25895 hypothetical protein
FLJ14996 35.3 418969 W33191 Hs 28907 hypothetical protein FLJ20258 SH3 35.2
453028 AB006532 Hs 31442 RecQ protein-like 4 DEAD, helicase_C 28.2 407722
BE252241 Hs 38041 pyridoxal (pyridoxine, vitamin pfkB 28.2 451721 NM_006946
Hs 26915 spectrin, beta, non-erythrocyt spectrin, PH, CH 27.9 416819 U77735
Hs 80205 pim-2 oncogene pkinase 27.9 430397 AI924533 Hs 105607 bicarbonate
transporter relate HCO3_cotransp 27.7 450334 AF035959 Hs 24879 phosphatidic
acid phosphatase PAP2 26.7 418945 BE246762 Hs 89499 arachidonate
5-lipoxygenase lipoxygenase, PLAT 25.3 424420 BE614743 Hs.146688 prostaglandin
E synthase MAPEG 25.1 412674 X04106 Hs 74451 calpain 4, small subunit (30 K)
efhand 24.4 430023 AA158243 Hs 227729 FK506-binding protein 2 (13 kD) FKBP
24.3 444672 Z95636 Hs 11669 laminin, alpha 5 laminin_EGF, laminin_G, EGF 24.0
413726 AJ278465 Hs 75510 annexin A11 annexin 23.1 438951 U51336 Hs 6453
inositol 1,3,4-triphosphate 5/ oxidored_nitro 23.0 429099 BE439952 Hs.196177
phosphorylase kinase, gamma 2 pkinase 23.0 431765 AF124249 Hs 268541 novel
SH2-containing protein 1 SH2 22.4 422645 L40027 Hs 118890 glycogen synthase
kinase 3 alp pkinase 22.4 413436 AF238083 Hs 68061 sphingosine kinase 1 DAGKc
22.3 422639 AI929377 Hs 173724 creatine kinase, brain ATP-gua_Ptrans,
ATP-gua_Pt 21.5 429869 AI907018 Hs.15977 Target CAT 21.3 418891 NM_002419 Hs
89449 mitogen-activated protein kina SH3, pkinase, pyridoxal_deC 21.1 419138
U48508 Hs 89631 ryanodine receptor 1 (skeletal RYDR_ITPR, RyR, SPRY, ion_tr
21.0 432866 BE395875 Hs 279609 mitochondrial carrier homolog mito_carr 20.9
452875 BE275760 Hs 30928 DNA segment on chromosome 19 (Euk_porin 20.8 426997
BE620738 Hs 173125 peptidylprolyl isomerase F (cy pro_isomerase 20.8 402916
ENSP00000202587* Bicarbonate t HCO3_cotransp 20.8 425760 D17629 Hs.159479
galactosamine (N-acetyl)-6-sul Sulfatase 20.7 400419 AF084545 Target EGF, ig,
lectin_c, sushi, Xli 20.0 419444 NM_002496 Hs 90443 Target CAT fer4 19.5
459133 U40343 Hs 29656 cyclin-dependent kinase inhibi ank 19.2 447595
AW379130 Hs 18953 phosphodiesterase 9A PDEase 19.2 422708 AB017430 Hs.119324
kinesin-like 4 kinesin, homeobox 19.0 414837 U24266 Hs.77448 aldehyde
dehydrogenase 4 famil aldedh 18.8 429712 AW245825 Hs.211914 ENSP00000233627*
NADH-ubiquino oxidored_q6 18.5 425848 BE242709 Hs 159637 valyl-tRNA
synthetase 2 GST_C, GST_N, Tropomyosin 18.4 451643 M64437 Hs 234799 breakpoint
cluster region RhoGEF, RhoGAP, PH, C2 18.1 447859 AK002194 Hs 19851
peroxisomal biogenesis factor 17.5 426457 AW894667 Hs 169965 chimerin
(chimaerin) 1 DAG_PE-bind, RhoGAP 17.3 421612 AF161254 Hs.106196 8D6 antigen
Idl_recept_a 17.1 421363 NM_001381 Hs 103854 docking protein 1, 62 kD (downs
PH, IRS 16.9 442739 NM_007274 Hs 8679 cytosolic acyl coenzyme A thio
Acyl-CoA_hydro 16.8 420568 F09247 Hs 247735 protocadherin alpha 10 cadherin
16.8 421445 AA913059 Hs.104433 Homo sapiens, clone IMAGE 4054 asp 16.8
425424 NM_004954 Hs 157199 ELKL motif kinase pkinase, KA1, UBA 16.7 446329
NM_013272 Hs 14805 solute carrier family 21 (orga kazal, OATP_N, OATP_C 16.5
406620 M81105 Hs 146550 myosin, heavy polypeptide 9, n myosin_head,
Myosin_tail, I 16.4 429109 AL008637 Hs 196352 neutrophil cytosolic factor 4
PX, SH3, OPR 16.3 429183 AB014604 Hs 197955 KIAA0704 protein PH,
Oxysterol_BP 16.2 444664 N26362 Hs 11615 map kinase phosphatase-like pr DSPc,
Rhodanese 16.2 427640 AF058293 Hs.180015 D-dopachrome tautomerase MIF,
late_protein_L2 16.2 425123 AW205274 Hs.154695 phosphomannomutase 2 PMM 16.0
416006 AA324251 Hs 78950 branched chain keto acid dehyd E1_dehydrog 15.8
412942 AL120344 Hs 75074 mitogen-activated protein kina pkinase 15.8 423366
Z80345 Hs.127610 acyl-Coenzyme A dehydrogenase, Acyl-CoA_dh, Acyl-CoA_dh_M
15.7 426391 AW161050 Hs 169611 second mitochondna-derived ac 15.7 424568

AF005418 Hs 150595 cytochrome P450, subfamily XXV p450 15.5 420029 BE258876 Hs 94446 polyamine-modulated factor 1 aldo_ket_red 15.5 433573 AF234887 Hs 57652 cadherin, EGF LAG seven-pass G 7tm_2, EGF, cadherin, lamini 15.4 407619 AL050341 Hs 37165 collagen, type IX, alpha 2 Collagen 15.3 427326 AI287878 gb qv23f06x1 NCI_CGAP_Lym6 Ho 7tm_1 15.2 442620 C00138 Hs 8535 Homo sapiens mRNA for KIAA1668 15.1 458130 AA115811 Hs.6838 ras homolog gene family, membe ras, arf 15.0 449936 AA938293 Hs 60088 hypothetical protein MGC11314 15.0 409230 AA852431 Hs 51299 NM_021074 Homo sapiens NADH de complex1_24 kD 14.7 423801 NM_015071 Hs.132942 GTPase regulator associated WI RhoGAP, SH3, PH 14.0 419639 AK001502 Hs 91753 hypothetical protein 13.6 419298 AA853479 Hs 89890 pyruvate carboxylase CPSase_L_chain, PYC_OADA, H 43.6 426108 AA622037 Hs 166468 programmed cell death 5 DUF122 13.5 448133 AA723157 Hs 73769 folate receptor 1 (adult) Folate_rec 13.5 418736 T18979 Hs 87908 Snf2-related CBP activator pro helicase_C, AT_hook 13.5 436543 NM_002212 Hs.5215 integrin beta 4 binding protei elF6 13.3 431515 NM_012152 Hs 258583 endothetial differentiation, l 7tm_1 13.3 429469 M64590 Hs 27 glycine dehydrogenase (decarbo GDC-P 13.2 431462 AW583672 Hs 256311 granin-like neuroendocrine pep 13.2 444855 BE409261 Hs 12084 Tu translation elongation fact GTR_EFTU, GTP_EFTU_D3, GTP.sub.-- 13.2 423464 NM_016240 Hs 128856 CSR1 protein Collagen 13.1 450787 AB006190 Hs 25475 aquaporin 7 MIP 13.0 428539 AW410063 Hs 184877 solute carrier family 25 (mito mito_carr 13.0 436014 AF281134 Hs 283741 exosome component Rrp46 RNaae_PH, RNase_PH_C 12.9 416866 AA297356 Hs.80324 serine/threonine protein phosp Metallophos 12.9 433867 AK000596 Hs 3618 hippocalcin-like 1 efhand 12.9 411408 U76666 Hs 69949 calcium channel, voltage-depen ion_trans 12.8 432329 NM_002962 Hs 2960 S100 calcium-binding protein A S_100, efhand 12.7 447887 AA114050 Hs.19949 caspase 8, apoptosis-related c ICE_p20, DED, ICE_p10 12.7 427448 BE246449 Hs 2157 Wiskott-Aldrich syndrome (ecze WH1, PBD, WH2 12.7 428820 AA436187 Hs 172631 integrin, alpha M (complement FG-GAP 12.7 446603 NM_014835 Hs 15519 oxysterol-binding protein-rela Oxysterol_BP 12.6 422633 X56832 Hs 118804 enolase 3, (beta, muscle) enolase 12.6 446839 BE091926 Hs.16244 mitotic spindle coiled-coil re Troponin 12.6 414757 U46922 Hs.77252 fragile histidine triad gene HIT 12.5 428593 AW207440 Hs 185973 degenerative spermatocyte (hom 12.5 432370 AA308334 Hs 274424 N-acetylneuraminic acid phosph Antifreeze, NeuB 12.5 401542 C15001413* gi.vertline.10645199.vertline.ref.vertline.NP.sub.-- 12.4 428782 X12830 Hs.193400 interleukin 6 receptor fri3, ig 12.3 425999 AW513051 Hs 332981 ESTs, Weakly similar to I38022 FAD_binding_2 12.3 422301 AI752163 Hs 114599 collagen, type VIII, alpha 1 C1q, Collagen 12.2 410720 AF035154 Hs 65756 regulator of G-protein signall RGS, G-gamma, DEP 12.2 407143 C14076 Hs.332329 EST 12.1 421321 NM_005309 Hs 103502 glutamic-pyruvate transaminase aminotran_1_2 12.1 425251 Z22521 Hs 155342 protein kinase C, delta pkinase, DAG_PE-bind, pkina 12.0 431354 BE046956 Hs 251673 DNA (cytoaine-5-)-methyltransf PWWP, PHD 12.0 420421 AF281133 Hs 343589 exosome component Rrp41 RNase_PH, RNase_PH_C 12.0 416714 AF283770 Hs 79630 CD79A antigen (immunoglobulin- ig, ITAM, Zn_clus 12.0 427336 NM_005658 Hs 2134 TNF receptor-associated factor MATH 12.0 409799 D11928 Hs.76845 phosphoserine phosphatase-like Hydrolase 11.9 436319 H90727 Hs 5123 inorganic pyrophosphatase Pyrophosphatase 11.9 400748 NM_022122 Homo sapiens matrix 11.9 428948 BE514362 FK506-binding protein 3 (25 kD) FKBP, PIP5K 11.8 401215 C12000457* gi.vertline.7512178.vertline.pir.vertline..vertline.T30 trypsin 11.7 401281 DKFZP586N2124 protein 11.7 427397 AI929685 Hs 177656 calmodulin 1 (phosphorylase ki efhand, RrnaAD 11.7 453496 AA442103 Hs 33084 solute carrier family 2 (facil sugar_tr 11.7 409608 AF231023 Hs.55173 cadherin, EGF LAG seven-pass G 7tm_2, cadherin, GPS, lamini 11.7 424415 NM_001975 Hs 146580 enolase 2, (gamma, neuronal) enolase 11.7 447495 AW401864 Hs 18720 programmed cell death 8 (apopt pyr_redox 11.6 426928 AF037062 Hs 172914 retinol dehydrogenase 5(11-ci adh_short 11.6 405371 NM_005569* Homo sapiens LIM do pkinase, LIM, PDZ 11.5 416282 R86664 Hs 167257 brain link protein-1 Xlink

11.4 452295 BE379936 Hs 28866 programmed cell death 10 11.4 430390 AB023186 Hs 241161 KIAA0969 protein PH 11.4 430594 AK000790 Hs 246885 hypothetical protein FLJ20783 PH 11.2 443814 BE281240 Hs 9857 carbonyl reductase 11.2 440242 AW295871 glucose transporter protein 10 11.1 447365 BE383676 Hs 334 Rho guanine nucleotide exchang SH3, PH, RhoGEF 11.1 400843 NM_003105*: Homo sapiens sortil ldl_recept_a, fn3, ldl_rece 11.1 422418 AK001383 Hs 116385 hypothetical protein FLJ10521 RhoGEF 11.0 400232 NM_001895* Homo sapiens casein pkinase 10.9 426828 NM_000020 Hs 172670 activin A receptor type II-lik pkinase, Activin_recp 10.9 431157 AI823969 Hs 132678 ESTs MAPEG 10.8 422616 BE300330 Hs 118725 selenophosphate synthetase 2 AIRS, AIRS_C 10.8 406779 AA412048 Hs 279574 CGI-39 protein, cell death-reg 10.8 400389 AL135841 olfactory receptor, family 2, 7tm_1 10.8 402207 Target Exon A2M_N, A2M 10.8 435615 Y15065 Hs 4975 potassium vollage-gated charine ion_trans, KCNQ1_channel

Detail Description Table CWU - DETL (117):
45TABLE 17A Pkey ExAccn UniGene ID Unigene Title Pred Protein Dom. R1 421296 NM_002666 Hs 103253 perilipin perilipin, SS 37.8 437897 AA770561 Hs.146170 hypothetical protein FLJ22969 SS, TM, zf-DHHC 29.2 453028 AB006532 Hs 31442 RecQ protein-like 4 DEAD, helicase_C, Fork_head 27.6 441021 AW578716 Hs.7644 H1 histone family, member 2 27.2 422310 AA316622 Hs.98370 cytochrome P450, subfamily IIS SS, TM, pkinase, fn3, ig 26.5 454017 AW023617 Hs 347130 hypothetical protein FLJ22709 SS, TM, myosin_head, RA, DAG.sub.-- 25.9 438424 AI912498 Hs 25895 hypothetical protein FLJ14996 SS, TM 25.8 435017 AA336522 Hs 12854 angiotensin II, type I recepto 25.0 409518 BE384836 Hs 3454 KIAA1821 protein SS 23.3 410418 D31382 Hs 63325 transmembrane protease, serine SS, TM, ldl_recept_a, trypsi 22.8 439924 AI985897 Hs.125293 ESTs SS 22.7 446374 AA329256 Hs.24756 ESTs, Moderately similar to al 22.6 431773 BE409442 Hs 268557 pleckstrin homology-like domai PH, SS, LIM, Troponin 21.4 420839 AI792682 Hs.282960 hypothetical protein MGC10870 SS, DS, UPF0139, Glyco_hydro 21.4 413436 AF238083 Hs 68061 sphingosine kinase 1 DAGKc 21.2 424420 BE614743 Hs 146688 prostaglandin E synthase MAPEG, SS, TM, MAPEG 20.7 422645 L40027 Hs 118890 glycogen synthase kinase 3 alp pkinase, SS, Ets 20.7 436725 BE045223 Hs.136912 hypothetical protein MGC10796 20.4 422098 H03117 Hs.111497 similar to mouse neuronal prot TM 20.2 429556 AW139399 Hs 98988 ESTs SS, pkinase, PMP22_Claudin 20.1 434068 AA977935 Hs 127274 ESTs SS 20.0 423767 H18283 Hs 132753 F-box only protein 2 F-box, SS, F-box, HORMA 19.9 423652 AF052122 Hs 130712 Homo sapiens clone 23929 mRNA ABC1, SS, PID, PID 19.8 422179 AF091619 Hs 112667 dynein, axonemal, intermediate WD40, SS 19.3 441356 BE384361 Hs.182885 ESTs, Weakly similar to JC5024 SS, TM, ank 18.5 418969 W33191 Hs.28907 hypothetical protein FLJ20258 SH3, SH3 17.2 432631 H08379 Hs 165563 hypothetical protein DKFZp434N TM, DnaJ, UBA, ArfGap, homeob 17.2 439108 AW163034 Hs 6467 synaptogyrin 3 Synaptogyrin, SS, TM, PDZ, WD 17.2 451643 M64437 Hs.234799 breakpoint cluster region RhoGEF, RhoGAP, PH, C2 17.2 434518 H56995 Hs 37372 Homo sapiens DNA binding pepti SS 16.9 413244 AW955951 Hs 159265 kruppel-related zinc finger pr SS, TM, BTB, Pep_M12B_propep 16.3 456642 AW451623 Hs.109752 putative c-Myc-responsive 16.2 421612 AF161254 Hs 106196 8D6 antigen ldl_recept_a, SS, TM 16.0 456177 NM_012391 Hs.79414 prostate epithelium-specific E Ets, SAM_PNT 15.7 409261 BE315042 Hs 19210 hypothetical protein MGC11308 15.6 414837 U24266 Hs 77448 aldehyde dehydrogenase 4 famil aldedh 15.6 401278 Target Exon Band_41 15.4 444804 AI084452 Hs 22158 hypothetical protein FLJ21988 SS 15.4 406620 M81105 Hs 146550 myosin, heavy polypeptide 9, n myosin_head, Myosin_tail, I 15.1 421495 AI583067 Hs 149152 ESTs, Weakly similar to RHOP M 15.0 416893 AA455588 Hs.62406 hypothetical protein FLJ22573 SS, rrm, SS 15.0 442620 C00138 Hs 8535 Homo sapiens mRNA for KIAA1668 SS, RNA_pol_K 14.9 406901 M14624 gb: Human 4-beta-galactosyltran 14.6 416006 AA324251 Hs 78950 branched chain keto acid dehyd E1_dehydrog 14.6 455557 AW995839 gb QV4-BN0044-110200-108-h07 B Metallophos 14.4 416819 U77735 Hs 80205 pim-2 oncogene pkinase, SS, TM, OTU,

K_tetra 14.3 444441 AW613841 Hs 301394 hypothetical protein MGC3101 14.0
406918 M88357 gb Homo sapiens DNA-binding pr zf-C2H2, SS 14.0 407605 W03512 Hs
6479 hypothetical protein MGC13272 SS, Sema, pkinase, TIG, PSI, e 13.6 447304
Z98883 Hs 18079 phosphatidylinositol glycan, c SS, Peptidase_C2 13.6 402365
Target Exon SS, SS, TM, ig 13.4 407767 W15398 Hs.38628 hypothetical protein
SS, zf-CCCH 13.3 432931 AF174487 Hs.293753 Bcl-2-related ovarian killer p
12.7 439233 AA831893 Hs 292767 hypothetical protein FLJ23109 zf-C3HC4, TM,
Sulfate_trans 12.7 423801 NM_015071 Hs.132942 GTPase regulator associated wi
RhoGAP, SH3, PH 12.6 430397 AI924533 Hs 105607 bicarbonate transporter relate
HCO3_cotransp, SS, TM 12.6 411570 BE144584 Hs 314341 ESTs 12.5 400206 Eos
Control SS, SS, Glyco_tranf_43, COLF 12.3 457941 AI004525 Hs 14587 ESTs,
Weakly similar to AF1518 SS, TM, SS, TM 12.2 412674 X04106 Hs.74451 calpain
4, small subunit (30 K) efhand, SS, CAP_GLY 12.0 400460 C11002253*:
gi.vertline.129091.vertline.sp.vertline.P23267 SS, TM, SCAN, zf-C2H2, KRAB
12.0 417595 AA424317 Hs.6259 KIAA1698 protein SS, TM, Glyco_hydro_31, Glyc
11.6 428758 AA433988 Hs.98502 CA125 antigen; mucin 16 SS 11.5 424707
BE061914 Hs.10844 Homo sapiens cDNA FLJ14476 fis SS, SS, TM, Sema 11.5 444359
AI697160 Hs 143594 ESTs, Weakly similar to HS4L_H 11.5 435158 AW663317 Hs
65588 DAZ associated protein 1 rrm, SS, rrm 11.3 407688 W25317 Hs.37616
Human D9 splice variant B mRNA 11.3 450503 R35917 Hs 301338 hypothetical
protein FLJ12587 SS 11.2 427448 BE246449 Hs.2157 Wiskott-Aldrich syndrome
(ecze WH1, PBD, WH2, SS 11.2 406230 Target Exon 11.2 432143 AL040183 Hs
123484 Homo sapiens, clone IMAGE: 4178 SS, TM, cys_rich_FGFR 11.2 433573
AF234887 Hs.57652 cadherin, EGF LAG seven-pass G SS, TM, 7tm_2, EGF, cadherin,
11.1 413726 AJ278465 Hs.75510 annexin A11 annexin, SS, annexin 11.1 431974
AW972689 Hs.200934 ESTs bZIP 11.0 428167 AA770021 Hs.16332 ESTs SS, ig, fn3
11.0 450461 BE408081 Hs.46736 hypothetical protein FLJ23476 SS 10.9 412738
N34731 Hs 74562 siah binding protein 1, FBP in homeobox 10.9 445434 BE391690
Hs 9265 hypothetical protein FLJ20917 SS, PWWP, Exonuclease, lipoc 10.9
444008 BE544855 Hs 236572 ESTs, Weakly similar to SFR4_H SS, SS, SAC3_GANP
10.7 444410 BE387360 Hs 33719 ESTs, Moderately similar to S6 SS 10.6 444607
AW405635 Hs.293687 ESTs SS, PI-PLC-X, PH, PI-PLC-Y, C 10.6 404333 C7001735*
gi.vertline.7768636.vertline.d- bj.vertline.BAA95 vwd 10.5 401210 C12000519:
gi.vertline.7710046.vertline.ref.vertline.NP_05 10.5 434743 AI363410
ribosomal protein S18 SS, TM 10.4 434030 AW162336 Hs.3709 low molecular mass
ubiquinone- SS 10.4 450029 AW073380 Hs 267963 hypothetical protein FLJ10535
SS, Pyndox_oxidase, zf-C2H 10.4 439632 AW410714 Hs 334437 hypothetical protein
MGC4248 SS, TM, transmembrane4 10.3 438185 Y19188 Hs 320461 ESTs SS 10.2
432031 AF039196 Hs.272367 hairless protein (putative sin jmjC 10.2 405371
NM_005569* Homo sapiens LIM do pkinase, LIM, PDZ 10.1 456741 W37608 Hs 184492
ESTs SS, pkinase 10.1 458130 AA115811 Hs 6838 ras homolog gene family, membe
ras, arf 10.0 456977 AK000252 Hs 169758 hypothetical protein FLJ20245 10.0
420029 BE258876 Hs 94446 polyamine-modulated factor 1 aldo_ket_red, SS, TM,
gla 10.0 445625 BE246743 hypothetical protein FLJ22635 SS, TM 9.9 423366
Z80345 Hs 127610 acyl-Coenzyme A dehydrogenase, Acyl-CoA_dh, Acyl-CoA_dh_M 9.8
458216 AW024282 Hs.104938 hypothetical protein MGC15906 9.8 451721 NM_006946
Hs 26915 spectrin, beta, non-erythrocyt spectrin, PH, CH, SS, Peptida 9.7
421445 AA913059 Hs 104433 Homo sapiens, clone IMAGE 4054 asp, SS, TM,
ion_trans, K_tet 9.7 431354 BE046956 Hs 251673 DNA (cytosine-5-)-methyltransf
SS, PWWP, PHD 9.7 443780 NM_012068 Hs 9754 activating transcription facto
bZIP, NTP_transf_2, SS, TBC 9.7 448133 AA723157 Hs.73769 folate receptor 1
(adult) Folate_rec, SS 9.7 444202 AL031685 Hs 12785 KIAA0939 protein SS, TM,
Na_H_Exchanger, ABC2 9.7 427640 AF058293 Hs 180015 D-dopachrome tautomerase
MIF, late_protein_L2, SS, GS 9.6 419167 AI589535 Hs.94875 ESTs, Weakly
similar to A35363 SS 9.6 424618 L29472 Hs 1802 major histocompatibility compl
TM, ig, MHC_II_beta, SS, TM, A 9.6 427497 AW139476 Hs 31240 ESTs 9.6 420423
AA827718 Hs 88218 ESTs SS 9.6 414756 AW451101 Hs 159489 ESTs, Moderately
similar to JC hexokinase2, hexokinase 9.6 407893 BE408359 Hs.43621 Homo
sapiens, Similar to hypot SS, SS, arf, ras, fn3, ras 9.5 408294 BE141732 gb

QV0-HT0101-061099-032-e07 H Ammonium_transp 9.5 442232 AI357813 Hs 337460 ESTs, Weakly similar to A47582 SS, TM, TGFb_propeptide, TGF 9.4 416866 AA297356 Hs 80324 serine/threonine protein phosp Metallophos, Metallophos 9.4 419823 AW271708 Hs 118918 ESTs, Weakly similar to M2OM_H SS, TM 9.4 422625 AW504698 Hs.155976 cullin 4B SS, SS, Cullin, Cullin 9.3 401264 C18000090* gi.vertline.6678656.vertline.ref.vertline.NP_0 SS, laminin_Nterm, laminin.sub.-- 9.3 407507 U73799 gb Human dynactin mRNA, partia SS, TM, HCO3_cotransp, CAP_G 9.2 400833 C11000890: gi.vertline.3746443.vertline.gb.vertline.AAC639 SS, TM, 7tm_1 9.2 422064 AW452589 Hs 335742 ESTs TM 9.2 452434 D30934 Hs.29549 C-type lectin-like receptor-1 lectin_c, SS, TM 9.2 421363 NM_001381 Hs 103854 docking protein 1, 62 kD (downs PH, IRS, TM, PH, IRS, trypsin, 9.1 427397 AI929685 Hs 177656 calmodulin 1 (phosphorylase ki efhand, RrnaAD, SS, efhand 9.1 431462 AW583672 Hs.256311 granin-like neuroendocrine pep SS 9.0 434796 AA812046 ESTs SS, myb_DNA-binding, myb_DN 9.0 422639 AI929377 Hs.173724 creatine kinase, brain ATP-gua_Ptrans, ATP-gua_Pt 9.0 447867 AI525268 Hs 164303 ESTs TM 9.0 442472 AW806859 gb MR0-ST0020-081199-004-c03 S SS, TM, Inos-1-P_synth, Occl 8.9 455588 AI129903 Hs 74669 vesicle-associated membrane pr synaptobrevin, SS, TM 8.9 454319 AW247736 Hs 101617 ESTs, Weakly similar to T32527 SS 8.9 429527 AA454184 Hs 289014 ESTs 8.9 432603 AA554920 Hs 105794 UDP-glucose glycoprotein gluco SS, TM 8.9 410338 W03445 Hs 38205 gb za05g11.r1 Soares melanocyt pkinase 8.9 452833 BE559681 Hs 30736 KIAA0124 protein WD40 8.9 407363 AF035032 Hs 181125 gb Homo sapiens clone MCA1L my SS, ig, SS, G_glu_transpept 8.8

PGPUB-DOCUMENT-NUMBER: 20030219782

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030219782 A1

TITLE: Compositions and methods for the modulation of sphingolipid metabolism and/or signaling

PUBLICATION-DATE: November 27, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Saba, Julie D.	Oakland	CA	US	
Fyrst, Henrik	Alameda	CA	US	

APPL-NO: 10/ 348052

DATE FILED: January 17, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60349582 20020117 US

US-CL-CURRENT: 435/6, 424/146.1 , 424/9.2 , 800/3 , 800/8

ABSTRACT:

Compositions, methods and kits for diagnosing and treating cancer and muscular disorders are provided. Therapeutic compositions may comprise agents that modulate sphingolipid metabolism and/or signaling pathways. Such compositions may be administered to a mammal afflicted with cancer. Diagnostic methods and kits may employ an agent suitable for detecting alterations in endogenous genes involved in sphingolipid metabolism. Such methods and kits may be used to detect the presence of a cancer or to evaluate the prognosis of a known disease. SPL polypeptides, polynucleotides and antibodies are also provided.

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of provisional application No. 60/349,582, filed Jan. 17, 2002 and U.S. application Ser. No. 10/053,510, filed Jan. 17, 2002, both applications incorporated herein by reference in their entirety.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (11):

[0012] Sphingosine-1-phosphate (S-1-P) is an endogenous sphingolipid metabolite present in most mammalian cells and in serum. Like other sphingolipid metabolites such as ceramide and sphingosine, S-1-P participates in specific signal transduction pathways. Many of the effects of S-1-P signaling, which include promotion of cellular proliferation, enhancement of migration, inhibition of apoptosis and stimulation of angiogenesis, influence the transformation, growth, drug resistance, vascularity and metastatic capacity of cancer cells. Several observations support the notion that sphingosine kinase (SK) and sphingosine-1-phosphate lyase (SPL) may be cancer related genes. First, the overexpression of SK in NIH3T3 fibroblasts leads to

oncogenic transformation as determined by the ability of transfected cells to form foci in vitro and to form fibrosarcomas in NOD/SCID mice. Second, human SPL was cloned and mapped to 10q21, a chromosomal region frequently deleted in a variety of human cancers. Taken together, these observations raise the possibility that SK and SPL may be potentially effective targets for pharmacological intervention in the treatment of cancer. Accordingly, the present invention provides methods for screening agents that modulate sphingolipid metabolism. Further, the present invention provides methods for detecting and treating cancer.

Summary of Invention Paragraph - BSTX (20):

[0020] Turning to another aspect, the present invention provides a method for identifying an agent that modulates sphingolipid metabolism, comprising (a) culturing a homozygous null mutant Drosophila melanogaster in the absence and presence of a candidate agent under conditions and for a time sufficient to observe in said mutant Drosophila melanogaster an effect of the agent on a level of either (i) at least one sphingolipid intermediate, or (ii) activity of at least one component of a sphingolipid pathway, wherein the mutant Drosophila melanogaster comprises a P-element transposon insertion in a gene encoding a component of a sphingolipid pathway that results in an altered activity level of at least one sphingolipid pathway component, and wherein the mutant Drosophila melanogaster exhibits a flightless phenotype that results from said insertion; and (b) comparing flight performance of the mutant Drosophila that is cultured in the presence of the candidate agent to the flight performance of the mutant Drosophila that is cultured in the absence of the candidate agent, wherein an increased flight performance of the mutant Drosophila cultured in the presence of the agent indicates the agent modulates sphingolipid metabolism. In certain embodiments the mutant Drosophila melanogaster comprises a homozygous mutation in a gene encoding a sphingosine-1-phosphate lyase (SPL), and in certain embodiments the homozygous null mutant Drosophila melanogaster comprises a T2 segment which comprises abnormal developmental patterning of thoracic muscles. In certain embodiments the agent that modulates sphingolipid metabolism inhibits sphingosine kinase activity.

Summary of Invention Paragraph - BSTX (21):

[0021] In yet another embodiment there is provided a method for identifying an agent that modulates sphingolipid signaling, comprising (a) culturing a homozygous null mutant Drosophila melanogaster in the absence and presence of a candidate agent under conditions and for a time sufficient to observe in said mutant Drosophila melanogaster an effect of the agent on a level of at least one sphingolipid intermediate, wherein the mutant Drosophila melanogaster comprises a P-element transposon insertion in a gene encoding a component of a sphingolipid pathway that results in an altered level of at least one sphingolipid intermediate; and (b) comparing the level of the sphingolipid intermediate that is generated in the presence of the candidate agent to the level in the absence of the candidate agent, wherein an altered level indicates the agent modulates sphingolipid signaling. It is also an aspect of the invention to provide an agent identified by the method of any one of the above described methods, which in certain embodiments is a composition comprising such agent in combination with a physiologically acceptable excipient. In certain embodiments there is provided a composition comprising an agent that increases flight performance in a homozygous null mutant Drosophila melanogaster, wherein the mutant Drosophila melanogaster comprises a P-element transposon insertion in a gene encoding a sphingosine-1-phosphate lyase (SPL) polypeptide that comprises the amino acid sequence set forth in SEQ ID NO:16, and wherein the mutant Drosophila melanogaster exhibits a flightless phenotype that results from said insertion, and in certain further embodiments the agent

inhibits sphingosine kinase activity.

Detail Description Paragraph - DETX (194):

[0233] The lace gene encodes one subunit of a Drosophila serine palmitoyltransferase. Inheritance of two lace.sup.k05305 null alleles is reported to be uniformly lethal, whereas the heterozygous allelic combination used in these experiments, lace.sup.k05305/lace.sup.2, leads to severe developmental phenotypes and a low percentage of viable progeny (Adachi-Yamada, T., T. Gotoh, I. Sugimura, M. Tateno, Y. Nishida, T. Onuki, and H. Date. 1999. Mol. Cell. Biol. 19: 7276-7286.). A Drosophila line homozygous for a null allele of one of two putative sphingosine kinase (SK) genes was also utilized in these experiments. This mutant (Sphk2.sup.KG05894) was created by the insertion of a P-element into the 5' UTR of CG2159, as previously described. The product of this gene functionally complements a yeast SK mutant. Wild type Canton-S (BL-1), lace.sup.2 (BL-3156), lace.sup.k05305 (BL-12176), and Sphk2.sup.KG05894 (BL-14133) lines were obtained from the Bloomington Drosophila Stock Center (Indiana University, Bloomington, Ind.).

PGPUB-DOCUMENT-NUMBER: 20030190650

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030190650 A1

TITLE: Screening method

PUBLICATION-DATE: October 9, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Cotter, Tom	Cork		IE	
Hayes, Ian	Cork		IE	
Murphy, Finbarr	Cork		IE	
Seery, Liam	Cork		IE	

APPL-NO: 10/ 332130

DATE FILED: May 19, 2003

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
GB	0016692.6	2000GB-0016692.6	July 7, 2000

PCT-DATA:
APPL-NO: PCT/GB01/03101
DATE-FILED: Jul 9, 2001
PUB-NO:
PUB-DATE:
371-DATE:
102(E)-DATE:

US-CL-CURRENT: 435/6, 435/7.23 , 514/12

ABSTRACT:

The present invention relates to a method for identifying a gene product which modulates the transition of a cell between a non-apoptopic state and an apoptopic state, comprising the steps of: (a) exposing the cell to an inhibitor of GM-CSF mediated inhibition of apoptosis; and (b) exposing the cell to one or more agents which increase tyrosine phosphorylation; and (c) C) placing the cell in conditions which permit it to undergo spontaneous apoptosis; and (d) monitoring the level(s) of expression of the one or more gene products in the cell; and (e) identifying gene product(s) whose expression has been increased, decreased or modified as a result of performing steps (a) to (d).

---------- KWIC ---------

Detail Description Table CWU - DETL (5):
5TABLE 4 A. UniGene Glio GM- Cluster Annotation T0 h 2 h CSF 2 h Hs.143212 cystatin F (leukocystatin) 0.59 0.64 1.63 Hs.78045 tissue factor pathway inhibitor 2 0.12 0.19 0.33 Hs.99932 diacylglycerol kinase, theta (110 kD) 0.62 0.79 1.33 Hs.40968 heparan sulfate (glucosamine) 3-O- 0.07 0.13 1 sulfotransferase 1 Hs.1499 heat shock transcription factor 1 0.1 0.09 0.4 Hs.111373 K1AA0423 protein 1.06 0.65 6 Hs.139120 ribonuclease P (30 kD) 0.69

3.2 2.72 Hs.36977 hemoglobin, delta 0.21 0.29 0.55 Hs.5105 ESTs 0.22 0.16 0.45 Hs.153678 reproduction 8 2.12 0.99 4.41 Hs.920 modulator recognition factor 0.75 0.57 2.19 Hs.13063 transcription factor CA150 0.15 0.18 0.36 Hs.813 ATPase, H+/K+ exchanging, beta 0.34 0.26 0.81 polypeptide Hs.194724 pronapsin A 0.3 0.87 2.16 Hs.48295 RNA helicase family 0.57 0.05 2.91 Hs.129078 ESTs 0.24 0.41 0.64 B. UniGene Glio GM- Cluster Annotation T0 h 4 h CSF 4 h Hs.227817 BCL2-related protein A1 0.1 0.18 0.72 Hs.12107 putative breast adenocarcinoma marker 1.11 1.39 2.49 (32 kD) Hs.101414 KIAA0557 protein 1 61 2 66 5.1 Hs.108014 tubulin, beta, 5 0.06 0.06 0.31 Hs.109281 Nef-associated factor 1 1.18 0.98 3.03 Hs.76307 neuroblastoma candidate region, 2.96 1.66 9.01 suppression of tumorigenicity 1 Hs.123233 ESTs 0.54 0.25 1.5 Hs.248038 major histocompatibility complex, class I, C 5.11 5.6 17.16 Hs.44512 ESTs 7.52 15.44 17.07 Hs.159494 Bruton agammaglobulinemia tyrosine kinase 0.17 0.13 0.4 Hs.104624 aquaporin 9 0.29 0.17 0.67 Hs.44197 Homo sapiens mRNA; cDNA DKFZp564D0462 2 3.48 4.15 (from clone DKFZp564D0462) Hs.80706 diaphorase (NADH/NADPH) (cytochrome b-5 4.99 6.34 13/79 reductase) Hs.167740 butyrophillin, subfamily 3, member A1 1.25 2.09 2.69 Hs.77897 pre-mRNA splicing factor SF3a (60 kD), similar 3.61 4.78 7.85 to S. cerevisiae PRP9 (spliceosome- associated protein 61) Hs.198278 6-phosphofructo-2-kinase/fructose-2,6- 0.12 0.25 1.13 biphosphatease 4 Hs.75607 myristoylated alanine-rich protein kinase C 3.86 2.93 8.8 substrate (MARCKS,80 K-L) Hs.169610 CD44 antigen (homing function and Indian 0.8 0.89 1.64 blood group system) Hs.44070 ESTs 12.57 14.1 43.43 Hs.56562 ESTs 10.45 18.98 28.91 Hs.239138 pre-B-cell colon-enhancing factor 1.02 0.87 2.41 Hs.177781 superoxide dismutase 2, mitochondrial 0.61 1.02 5.28 Hs.111373 KIAA0423 protein 1.33 0.9 5.03 Hs.104481 Homo sapiens mRNA for Nck, Ash and 6.42 13.83 14.54 phospholipase C gamma-binding protein NAP4, partial cds Hs.242908 lecithin-cholesterol acyltransferase 3.69 5.74 11.01 Hs.153 ribosomal rotein L7 0.03 0.28 0.71 Hs.179600 TAP binding protein (tapasin) 1.82 1.38 5.64 Hs.2561 nerve growth factor, beta polypeptide 0.41 0.35 2.36 Hs.82212 CD53 antigen 2.38 3.13 5.29 Hs.150580 putative translation initiation factor 1.76 1.73 5.1 Hs.2064 vimentin 0.66 0.5 2.11 Hs.81361 heterogeneous nuclear ribonucleoprotein A/B 14.18 4.04 35.31 Hs.119252 tumor protein, translationally-controlled 1 1.95 1.5 9.33 Hs.75372 N-acetlgalactosaminidase, alpha- 1.08 1.79 2.49 Hs.68061 ESTs, Weakly similar to sphingosine kinase 0.4 0.43 1.08 [M. musculus] Hs.74122 caspase 4, apoptosis-related cysteine 0.73 0.67 1.47 protease Hs.18350 chromosome 21 open reading frame 1 2.81 3.94 7.37 Hs.2488 lymphocyte cytosolic protein 2 (SH2 domain- 1.06 0.78 2.3 containing leukocyte protein of 76 kD) Hs.60177 KIAA0996 protein 3.8 7.44 7.75 Hs.184592 KIAA0344 gene product 2.23 4.17 10.16 Hs.76452 C-reactive protein, pentraxin-related 0.36 0.15 0.78 Hs.753 formyl peptide receptor 1 1.03 1.01 2.44 Hs.33540 ESTs, Weakly similar to KIAA0765 protein 5.26 3.08 11.53 [H. sapiens] Hs.79018 chromatin assembly factor 1, subunit A 34.29 65.93 81.83 (p150) Hs.22176 ESTs 118.49 78.07 260.42 Hs.51077 Integrin, alpha X (antigen CD11C (p150), alpha 2.62 4.09 6.92 polypeptide) Hs.5392 Homo sapiens clone 25030 mRNA sequence 0.7 0.72 4 Hs.813 ATPase, H+/K+ exchanging, beta polypeptide 0.34 0.3 3.15 Hs.62954 ferritin, heavy polypeptide 1 0.24 0.25 2.1 Hs.239124 ESTs 1.88 4.61 3.96 Hs.194724 pronapsin A 0.3 0.44 5.97 Hs.21812 ESTs 3.4 6.45 6.87 Hs.129078 ESTs 0.24 0.38 1.12 C. UniGene Glio GM- Cluster Annotation T0 h 6 h CSF 6 h Hs.143212 cystatin F (leukocystatin) 0.59 0.17 1.93 Hs.90370 actin related protein 213 complex, subunit 1A 0.08 0.13 0.34 (41 kD) Hs.10762 ESTs 0.23 0.23 0.49 Hs.78045 tissue factor pathway inhibitor 2 0.12 0.21 0.34 Hs.24930 tubulin-specific chaperane a 0.09 0.09 0.47 Hs.227817 BCL2-related protein A1 0.08 0.15 0.33 Hs.17518 Homo sapiens cig5 mRNA, partial sequence 0.58 0.5 1.23 Hs.109281 Nef-associated factor 1 1.18 0.97 3.01 Hs.76807 Human HLA-DR alpha-chain mRNA 0.24 0.29 0.53 Hs.44287 ESTs 0.26 0.28 0.9 Hs.104624 aquaporin 90.24 0.26 0.55 Hs.31314 retinoblastoma-binding protein 7 0.13 0.17 0.35 Hs.114138 ESTs 2.72 7.41 9.22 Hs.194562 telomeric repeat binding factor

(NIMA 0.26 0.28 0.67 interacting) 1 Hs.208819 ESTs 0.06 0.04 0.64 Hs.82128
5T4 oncofetal trophoblast glycoprotein 0.85 0.57 1.81 Hs.19126 src
kinase-associated phosphoprotein of 55 0.13 0.33 0.3 kDa Hs.198278
6-phosphofructo-2-kinase/fructose-2,6- 0.12 0.11 0.48 biphosphatase 4
Hs.154583 RNA-binding protein S1-1, human homolog 0.17 0.21 0.44 of Hs.72964
makorin, ring finger protein, 3 0.15 0.2 0.44 Hs.133554 ESTs 0.37 0.5 0.76
Hs.159509 alpha-2-plasmin Inhibitor 0.04 0.04 0.3 Hs.98183 ESTs 0.15 0.22 0.4
Hs.41683 cartilage paired-class homeoprotein 1 0.15 0.25 0.4 Hs.155995
KIAA0643 protein 0.17 0.3 0.4 Hs.2062 vitamin D (1,25-dihydroxyvitamin D3)
0.61 0.87 1.39 receptor Hs.51299 NADH dehydrogenase (ubiquinone) 0.36 0.3
0.97 flavoprotein 2 (24 kD) Hs.170180 sialyltransferase 8 alpha-2,8- 0.16
0.14 0.84 polysialytransferase D Hs.177781 superoxide dasmutase 2,
mitochondrial 0.61 0.67 3.19 Hs.75789 N-myc downstream regulated 0.18 0.19
0.36 1 Hs.179600 TAP binding protein (tapasin) 1.82 1.48 3.69 Hs.100407
programmed cell death 4 0.39 0.56 0.81 Hs.103755 receptor-interacting
serine-threonine kinase 0.19 0.08 0.4 2 Hs.123141 Wiskott-Aldrich
syndrome-like 0.11 0.12 0.51 Hs.2064 vimentin 0.66 0.66 1.48 Hs.119252 tumor
protein, translationally-controlled 1 1.77 2.22 4.35 Hs.13957 ESTs 0.24 0.26
0.51 Hs.227133 KIAA0670 protein 0.13 0.08 0.3 Hs.28169 KIAA0459 protein 0.77
0.68 2.55 Hs.108947 KIAA0050 gene product 0.31 0.08 1.47 Hs.68061 ESTs,
Weakly similar to sphangosine kinase 0.4 0.4 1.01 [M. musculus] Hs.44426
ESTs, Weakly similar to PHOSPHOLIPID 0.59 0.67 1.39 HYDROPEROXIDE GLUTATHIONE
PEROXIDASE [H. sapiens] Hs.251972 complement component 3 0.21 0.22 0.46
Hs.927 myosin-binding protein H 0.17 0.31 0.34 Hs.13063 transcription factor
CA150 0.1 0.26 0.3 Hs.113216 oxytocin, prepro- (neurophysin I) 0.13 0.04 0.31
Hs.62954 ferritin, heavy polypeptide 1 0.22 0.22 1.24 Hs.194724 pronapsin A
0.3 0.16 0.8 Hs.235069 RecQ protein-like DNA helicase Q1-like 1.16 2.23 2.71
Hs.124380 ESTs 0.52 0.57 1.8 Hs.241510 interferon-induced protein 41, 30 kD
0.35 0.34 0.72 Hs.129078 ESTs 0.24 0.14 1.16

PGPUB-DOCUMENT-NUMBER: 20030175939

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030175939 A1

TITLE: Sphingosine-1-phosphate lyase polypeptides, polynucleotides and modulating agents and methods of use therefor

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Saba, Julie D.	Oakland	CA	US	
Fyrst, Henrik	Alameda	CA	US	

APPL-NO: 10/ 053510

DATE FILED: January 17, 2002

RELATED-US-APPL-DATA:

child 10053510 A1 20020117

parent continuation-in-part-of 09356643 19990719 US GRANTED

parent-patent 6569666 US

child 09356643 19990719 US

parent continuation-in-part-of 08939309 19970929 US GRANTED

parent-patent 6423527 US

US-CL-CURRENT: 435/232, 435/18 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

Compositions, methods and kits for diagnosing and treating cancer are provided. Therapeutic compositions may comprise agents that modulate the expression or activity of a sphingosine-1-phosphate lyase (SPL). Such compositions may be administered to a mammal afflicted with cancer. Diagnostic methods and kits may employ an agent suitable for detecting alterations in endogenous SPL. Such methods and kits may be used to detect the presence of a cancer or to evaluate the prognosis of a known disease. SPL polypeptides, polynucleotides and antibodies are also provided.

--------- KWIC ---------

Summary of Invention Paragraph - BSTX (52):

[0048] As noted above, the present invention is generally directed to compositions and methods for the diagnosis and therapy of cancers such as breast cancer. The invention is more particularly related to sphingosine-1-phosphate lyase (SPL) polypeptides, which have the ability to cleave sphingosine-1-phosphate into inactive metabolites, and to polynucleotides encoding such polypeptides. Sphingosine-1-phosphate (S-1-P) is

an endogenous sphingolipid metabolite present in most mammalian cells and in serum. Like other sphingolipid metabolites such as ceramide and sphingosine, S-1-P participates in specific signal transduction pathways. The results of S-1-P signaling are diverse and dependent upon the cell type being examined. However, many of the effects of S-1-P signaling, which include promotion of cellular proliferation, enhancement of migration, inhibition of apoptosis and stimulation of angiogenesis, influence the transformation, growth, drug resistance, vascularity and metastatic capacity of cancer cells. The gene encoding the enzyme responsible for S-1-P synthesis is sphingosine kinase, SK, and S-1-P degradation is sphingosine phosphate lyase, SPL and S-1-P phosphatase, S-1-PP. Several observations support the notion that SPL may be a cancer related gene. First, altered expression of SPL in human tumors compared to corresponding normal tissue from the same patient has been shown. Second, human SPL maps to 10q21, a chromosomal region frequently deleted in a variety of human cancers. Taken together, these observations raise the possibility that SPL may be potentially effective targets for pharmacological intervention in the treatment of cancer.

PGPUB-DOCUMENT-NUMBER: 20030162692

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030162692 A1

TITLE: Follicle stimulating hormone stimulated genes and uses thereof

PUBLICATION-DATE: August 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Wong, Grace	Brookline	MA	US	

APPL-NO: 09/ 854434

DATE FILED: May 11, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60203805 20000512 US

US-CL-CURRENT: 514/2, 435/4 , 435/6

ABSTRACT:

The present invention is directed to the identification genes that are expressed at a higher level in certain FSH or FSH Mimetic treated cells than in otherwise identical untreated cells. Genes that are expressed at a higher level in FSH or FSH Mimetic treated cells than untreated cells ("FSH or FSH Mimetic stimulated genes") are of interest, in part, because FSH or FSH Mimetics can or could influence a wide range of cellular processes and responses in reproduction, including steroidogenesis and gamatogenesis. The identified FSH or FSH Mimetic stimulated genes and the proteins they encode can be used: 1) as therapeutic agents which modulate a cellular process or response that is influenced by FSH or FSH Mimetic; 2) as targets for use in high throughput screening and the development of therapeutic agents which modulate a cellular process or response that is influenced by FSH or FSH Mimetic; and 3) as markers which can be used to detect and monitor a cellular process or response that is influenced by FSH or FSH Mimetic.

[0001] This application claims the benefit of U.S. Provisional Application No. 60/203,805, filed May 12, 2000.

---------- KWIC ---------

Detail Description Table CWU - DETL (5):
4TABLE 4 Forskolin 16 hr time point 106 genes upregulated > = 2-fold 022DAAMH Fold Cont Forsk Gene name Acc # 2.1 1661 3449 antigen identified by monoclonal antibodies 4F2 [IMAGE:478301] AA049696.1 4.1 183 750 carbonic anhydrase 6 [IMAGE:329940] AI327498.1 2.4 227 552 chaperonin subunit 4 (delta) [IMAGE:459668] AA027583.1 2.1 259 545 chondroitin sulfate proteoglycan 2 [IMAGE:355990] W49048.1 2.1 624 1320 ESTs [IMAGE:315676] W09957.1 2 424 859 ESTs [IMAGE:316914] W11926.1 2 939 1885 ESTs

[IMAGE:317466] W34061.1 3.7 448 1660 ESTs [IMAGE:350182] W34722.1 2.7 374 1004 ESTs [IMAGE:367445] W50706.1 2.3 512 1203 ESTs [IMAGE:372421] W53621.1 2 396 783 ESTs [IMAGE:386218] W65070.1 2 85 169 ESTs [IMAGE:403166] W82868.1 2 378 769 ESTs [IMAGE:404057] W82577.1 2.5 106 270 ESTs [IMAGE:424848] W98118.1 3.8 303 1150 ESTs [IMAGE:426033] AA002836.1 2.4 140 339 ESTs [IMAGE:427480] AA002452.1 2.1 365 761 ESTs [IMAGE:466678] AA031159.1 2.8 272 757 ESTs [IMAGE:477003] AA048121.1 2.3 154 352 ESTs [IMAGE:479247] AA048730.1 6.2 209 1295 ESTs [IMAGE:482641] AA061982.1 2.2 387 834 ESTs [IMAGE:483476] AA060036.1 2.3 310 709 ESTs [IMAGE:483649] AA061366.1 2.3 374 859 ESTs [IMAGE:572819] AA110791.1 8.6 58 498 ESTs [IMAGE:598824] AA168416.1 2.5 101 251 ESTs [IMAGE:620209] AA177920.1 2.1 246 527 ESTs [IMAGE:622893] AA177702.1 2 238 469 ESTs [IMAGE:636730] AA189425.1 2.5 114 289 ESTs [IMAGE:640085] AA198542.1 3.8 324 1223 ESTs [IMAGE:656089] AA239554.1 2.2 258 564 ESTs [IMAGE:680250] AA237600.1 2.7 52 139 ESTs [IMAGE:749313] AA288555.1 2 121 236 ESTs [IMAGE:762306] AA277421.1 2.7 226 607 ESTs [IMAGE:790122] AA387971.1 4.7 59 278 ESTs [IMAGE:818790] AA467382.1 2.3 120 274 ESTs [IMAGE:876063] AA475435.1 4 149 602 ESTs, Highly similar to CYSTATHIONINE GAMMA-LYASE [Homo sap AA096870.1 3.4 52 175 ESTs, Highly similar to CYSTATHIONINE GAMMA-LYASE [Homo sap AA245993.1 2 683 1346 ESTs, Highly similar to DELTA 1-PYRROLINE-5-CARBOXYLATE SYN W41878.1 2.7 200 531 ESTs, Highly similar to GLYPICAN-3 PRECURSOR [Rattus norvegicu AA274932.1 2.1 506 1072 ESTs, Highly similar to HAM1 PROTEIN [Saccharomyces cervisiae] W34474.1 2 372 761 ESTs, Highly similar to HYPOTHETICAL 25.7 KD PROTEIN IN MSH1- W54688.1 2.3 311 708 ESTs, Highly similar to REPLICATION PROTEIN A 14 KD SUBUNIT [H AA000318.1 2 304 602 ESTs, Highly similar to SERINE HYDROXYMETHYLTRANSFERASE, AA208877.1 11.6 916 10602 ESTs, Highly similar to acid ceramidase [M. musculus] [IMAGE:76308 AA286605.1 2.1 574 1206 ESTs, Highly similar to CGI-81 protein [H. sapiens] [IMAGE:467379] AA036624.1 2 224 443 ESTs, Highly similar to HSPC040 protein [H. sapiens] [IMAGE:332442] W08432.1 2.1 657 1358 ESTs, Highly similar to probable calcium-binding protein [H. sapiens] W18735.1 2.2 377 828 ESTs, Highly similar to similar to Schizosaccharomyces pombe splic W11916.1 2.1 359 744 ESTs, Moderately similar to NADH-UBIQUINONE OXIDOREDUCTASE W97248.1 3.2 715 2301 ESTs, Weakly similar to cDNA EST EMBL:D75506 comes from this ge W16247.1 2.2 658 1423 ESTs, Weakly similar to GARP PROTEIN PRECURSOR [H. sapiens] [IM AA048874.1 2.6 245 642 ESTs, Weakly similar to heat shock protein hsp40-3 [M. musculus] [IM AA049615.1 2.7 1071 2916 ESTs, Weakly similar to HISTIDINE-RICH PROTEIN KE4 [M. musculus] W18585.1 2.5 218 551 ESTs, Weakly similar to HYPOTHETICAL 11.4 KD PROTEIN C13G6.04 W11535.1 2 161 330 ESTs, Weakly similar to HYPOTHETICAL 11.4 KD PROTEIN C13G6.04 AA116946.1 2.3 300 698 ESTs, Weakly similar to LYMPHOCYTE ANTIGEN LY-6A.2/LY-6E.1 PR AA472994.1 2.1 124 263 ESTs, Weakly similar to mCAC [M. musculus] [IMAGE:350881] W40994.1 2.5 212 536 ESTs, Weakly similar to ORF YGL231c [S. cerevisiae] [IMAGE:442681] AA015149.1 2 177 359 ESTs, Weakly similar to putative [C. elegans] [IMAGE:571422] AA109015.1 2.3 724 1644 ESTs, Weakly similar to similar to leucyl-tRNA synthetase [C. elegans] W11665.1 2.9 1092 3190 ESTs, Weakly similar to similar to nucleotide translocator [C. elegans] AA213247.1 3.7 193 721 extracellular matrix protein 1 [IMAGE:678765] AA237378.1 7.4 369 2744 extracellular matrix protein 1 [IMAGE:874833] AA474897.1 2.9 497 1443 glutamate oxaloacetate transaminase 1, soluble [IMAGE:481381] AA060494.1 3 254 755 glutathione-S-transferase, alpha 3 [IMAGE:766582] AA274682.1 2.7 427 1169 glutathione-S-transferase, alpha 4 [IMAGE:367627] W54349.1 3 108 326 growth arrest specific 2 [IMAGE:820540] AA423395.1 3.2 198 627 heme oxygenase (decycling) 1 [IMAGE:677499] AA213167.1 2 291 570 histidine triad nucleotide-binding protein [IMAGE:533117] AA068901.1 2.3 283 658 hormone receptor [IMAGE:439773] AA008625.1 2 87 174 hormone receptor [IMAGE:641865] AA209882.1 2.6 482 1263 lectin, galactose binding, soluble 3 [IMAGE:717226] AA403841.1 3 608 1820 lymphocyte antigen 6 complex [IMAGE:580715] AA145865.1 3.6 371 1333 lymphocyte antigen 6 complex, locus C [IMAGE:425855] AA000712.1 2.1 312 640 male enhanced antigen 1 [IMAGE:463746]

AA028786.1 2.4 435 1058 metallothionein 1 [IMAGE:480068] AA051654.1 2.6 183 475 metallothionein 1 [IMAGE:480920] AA064247.1 2.2 324 718 Mouse chromatin nonhistone high mobility group protein (HGM-I(Y), AA538243.1 2.2 463 1033 Mouse mRNA for dbpA murine homologue, complete cds [IMAGE:481 AA059953.1 2 219 428 Mus musculus A10 mRNA, partial cds [IMAGE:333376] W15888.1 2.1 673 1403 Mus musculus A10 mRNA, partial cds [IMAGE:385441] W61383.1 2 646 1292 Mus musculus eIF-1A (eIF-1A) mRNA, complete cds [IMAGE:747322] AA274946.1 2.2 363 786 Mus musculus mRNA for HIRA-interacting protein (HIRIP5) [IMAGE:7 AA270607.1 2.6 344 878 Mus musculus mRNA for Sid1669p, complete cds [IMAGE:922965] AA511365.1 2.9 253 730 Mus musculus SH3-containing protein SH3P2 mRNA, partial cds [IMA AA024088.1 2.5 148 372 Mus musculus SH3-containing protein SH3P2 mRNA, partial cds [IMA AA166372.1 10.6 268 2833 myeloid differentiation primary response gene 116 [IMAGE:475803] AA050417.1 2.3 652 1470 myosin, heavy polypeptide 8, skeletal muscle, perinatal [IMAGE:3174 W13528.1 2 355 720 nuclear, factor, erythroid derived 2, like 2 [IMAGE:475505] AA044475.1 2 309 625 periplakin [IMAGE:571984] AA105152.1 2 65 128 protein kinase C, delta [IMAGE:421002] W91539.1 2.4 75 180 protein tyrosine phosphatase, non-receptor type 8 [IMAGE:574608] AI323214.1 2.2 122 265 Public domain EST [IMAGE:426378] AA002886.1 2.9 192 549 Public domain EST [IMAGE:437685] AA007828.1 7 184 1293 Public domain EST [IMAGE:467785] AA036495.1 2.3 108 246 Public domain EST [IMAGE:574227] AA119136.1 2.2 145 317 Public domain EST [IMAGE:681424] AA237757.1 3.2 69 223 Public domain EST [IMAGE:716713] AA265198.1 2 116 237 Public domain EST [IMAGE:805306] AA473329.1 2.3 337 765 Public domain EST [IMAGE:874030] AA472200.1 2 1049 2049 requiem [IMAGE:573346] AI323194.1 2.4 127 304 sphingosine kinase 1 [IMAGE:425961] AA000819.1 2 839 1677 split hand/foot deleted gene 1 [IMAGE:850971] AA462396.1 2.4 365 894 stimulated by retinoic acid 14 [IMAGE:480896] AA064241.1 2.1 256 530 TG interacting factor [IMAGE:722623] AA260654.1 3.4 227 761 tryptophanyl-tRNA synthetase [IMAGE:367765] W53959.1

PGPUB-DOCUMENT-NUMBER: 20030162206

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030162206 A1

TITLE: Ceramide kinase and DNA encoding it

PUBLICATION-DATE: August 28, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Sugiura, Masako	Tokyo		JP	
Kono, Keita	Kawasaki-shi		JP	
Kohama, Takafumi	Tokyo		JP	

APPL-NO: 10/ 315597

DATE FILED: December 10, 2002

RELATED-US-APPL-DATA:

child 10315597 A1 20021210

parent continuation-in-part-of PCT/JP01/04889 20010611 US UNKNOWN

FOREIGN-APPL-PRIORITY-DATA:

COUNTRY	APPL-NO	DOC-ID	APPL-DATE
JP	2000-178039	2000JP-2000-178039	June 14, 2000

US-CL-CURRENT: 435/6, 435/194 , 435/320.1 , 435/325 , 435/69.1 , 536/23.2

ABSTRACT:

A protein having ceramide kinase activity which can be a target for a prophylactic or therapeutic medicament against neuronal disease, inflammation, HIV infection, type 2 diabetes mellitus, obesity, septicemia, arteriosclerosis and cancer. Specifically, a protein which comprises an amino acid sequence shown in the amino acid numbers 1-537 of SEQ ID No. 2 of the Sequence Listing, a DNA which encodes the protein, a recombinant vector comprising the DNA, a host cell transformed with the recombinant vector and a method for producing the protein. By using the method of the present invention, a compound is provided having a specifically activating or inhibiting activity to ceramide kinase and is useful as a medicament for treating a neuronal disorder, an anti-inflammatory medicament, a medicament for treating HIV infection, an anti-type 2 diabetes mellitus medicament, an anti-obesity medicament, an anti-septicemia medicament, an anti-arteriosclerosis medicament and an anticancer medicament.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (13):

[0011] In addition, an expressed sequence tag (hereinafter referred to as "EST") corresponding to a part of the DNA which encodes the protein of the present invention is known, when a homology search is performed by using the dbEST database of NCBI (National Center for Biotechnology Information, U.S.A.) based on the sequence of known mouse sphingosine kinase. However, there is no

specific suggestion regarding the function of the protein of the present invention in said EST data.

Summary of Invention Paragraph - BSTX (37):

[0033] More specifically, for instance, part or all of the DNA which encodes the protein of the present invention can be obtained by preparing a probe based on the full sequence or a partial sequence of cDNA of mouse sphingosine kinase, and analyzing the sequence of the clones obtained from a cDNA library derived from mammals by performing a colony-hybridization method under the conditions of low stringency, and selecting the clone having a sequence highly homologous to the nucleotide sequence shown in the nucleotide numbers 124-1734 of SEQ ID No. 1 of the Sequence Listing. Moreover, a part of the DNA which encodes the protein of the present invention can be obtained also by preparing primers based on the known sequence of mouse sphingosine kinase (preferably primers are prepared based on the sequence of the conserved region in phylogeny), and performing a polymerase chain reaction (hereinafter referred to as "PCR", see Saiki R. K. et al., (1988), Science, 239, 487 to 491) using the cDNA library derived from mammals as a template, or performing reverse-transcriptase polymerase chain reaction (hereinafter referred to as "RT-PCR") using mRNA derived from mammals as a template, and analyzing the resultant sequence that the product has and selecting the clone having a sequence which is highly homologous to the nucleotide sequences shown in the nucleotide numbers 124-1734 of SEQ ID No. 1 of the Sequence Listing.

Detail Description Paragraph - DETX (3):

[0131] 1) Search for cDNA Clone Having Low Homology to Mouse Sphingosine Kinase 1 and Analysis of a Nucleotide Sequence

Detail Description Paragraph - DETX (4):

[0132] Homology search was performed by using the algorithm of tblastn to the dbEST database of NCBI based on the known amino acid sequence of mouse sphingosine kinase 1, and an EST clone (GenBank accesion number AA355581) which shares the homology with mouse sphingosine kinase I was found.

Detail Description Paragraph - DETX (21):

[0149] All the nucleotide sequences of cDNA inserted in plasmids pBK-29, pBK-5, and pBK-33 obtained by the above 4), were determined by the dideoxy nucleotide chain termination method. In addition, parts of the sequences were analyzed by using full automatic DNA sequence analysis apparatus (model 373A, manufactured by Perkin Elmer Japan Applied Biosystems corporation). Consequently, cDNA inserted in pBK-33 had the nucleotide sequence which consists of 4463 bp(s) (SEQ ID No. 1 of the Sequence Listing) including all of the sequences of the inserted cDNAs of pBK-29 and pBK-5. Furthermore, this nucleotide sequence and the amino acid sequence encoded by the open reading frame (hereinafter referred to as "ORF") included in this nucleotide sequence (amino acid numbers 1-537 of SEQ ID No. 2 of the Sequence Listing) were subjected to homology search with DNA databases, GenBank and EMBL, and a protein database, SWISS-PLOT. As a result of the homology search, there were no proteins having known function which were especially highly homologous to the amino acid sequence shown in the amino acid numbers 1-537 of SEQ ID No. 2 of the Sequence Listing, and thus it became clear that it is a novel protein. Moreover, the sequence homology between the amino acid sequence which is shown in the amino acid numbers 1-537 of SEQ ID No. 2 of the Sequence Listing and the amino acid sequence covering the full length of the human sphingosine kinase 1 or 2 was only 29%, but the conserved domain thereof was highly homologous to

those of sphingosine kinases beyond species (see T. Kohama et al., (1998), J. Biol. Chem., 273.23722-23728). Furthermore, when a motif search was performed, it was suggested that it possessed a PH domain (see A. D. Ma & C. S. Abrams, (1999), Thromb. Haemost, 82,399 to 406) and a diacylglycerol kinase domain (See F. Sakana & H. Kanoh, (1997), Int. J. Biochem. Cell Biol., 29, 1139-1143). The cDNA which encoded the novel protein thus obtained was named hCERK1.

PGPUB-DOCUMENT-NUMBER: 20030157086

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030157086 A1

TITLE: Protection of the female reproductive system from natural and artificial insults

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Tilly, Jonathan L.	Windham	NH	US	
Kolesnick, Richard N.	New York	NY	US	

APPL-NO: 10/ 217259

DATE FILED: August 12, 2002

RELATED-US-APPL-DATA:

child 10217259 A1 20020812

parent continuation-in-part-of 09503852 20000215 US PENDING

US-CL-CURRENT: 424/94.6

ABSTRACT:

Described are methods for protecting the female reproductive system against natural and artificial insults by administering to women a composition comprising an agent that antagonizes one or more acid sphingomyelinase (ASMase) gene products. Specifically, methods disclosed herein serve to protect women's germline from damage resulting from cancer therapy regimens including chemotherapy or radiotherapy. In one aspect, the method preserves, enhances, or revives ovarian function in women, by administering to women a composition containing sphingosine-1-phosphate, or an analog thereof. Also disclosed are methods to prevent or ameliorate menopausal syndromes and to improve in vitro fertilization techniques.

RELATED APPLICATION

[0001] The present application is a continuation-in-part of pending U.S. patent application Ser. No. 09/503,852, filed Feb. 15, 2000, and claims benefit thereof.

---------- KWIC --------

Summary of Invention Paragraph - BSTX (7):

[0007] Since the initial discovery ofthe sphingomyelin pathway, numerous studies have been published on the potential role of ceramide in signaling cell death (Hannun, (1996) id.; and Kolesnick & Kronke (1998) id.). A central role for ceramide, a pro-apoptotic sphingolipid (Kolesnick & Kronke, Annu. Rev. Physiol., 60:643 (1998)) derived from either sphingomyelin hydrolysis or de novo synthesis, in mediating death of oocytes exposed to anti-cancer therapies, has recently emerged (Perez et al, Nat. Med., 3:1228 (1997); and Morita, Y. et

al.,Nat. Med. 6, 1109-1114 (2000)). Oocyte apoptosis is suppressed by disruption of the acid sphingomyelinase gene or by sphingosine-1-phosphate therapy (Morita, Y. et al.,Nat. Med. 6, 1109-1114 (2000)). Whether or not cells die in response to ceramide elevations is, however, at least partly dependent upon the rate at which ceramide is metabolized. It is now known that ceramide can also be metabolized via ceramidase to sphingosine, which is then phosphorylated by sphingosine kinase to generate sphingosine-1-phosphate (S1P), a potent antagonist of ceramide-promoted apoptosis (Cuvillier et al., Nature 381, 800 (1996); Spiegel et al., Ann. N.Y. Acad. Sci 845, 11 (1998); and Spiegel, J. Leukoc. Biol. 65, 341 (1999)).

PGPUB-DOCUMENT-NUMBER: 20030157082

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030157082 A1

TITLE: Methods and compositions for treating cancer using 140, 1470, 1686, 2089, 2427, 3702, 5891, 6428, 7181, 7660, 25641, 69583, 49863, 8897, 1682, 17667, 9235, 3703, 14171, 10359, 1660, 1450, 18894, 2088, 32427, 2160, 9252, 9389, 1642, 85269, 10297, 1584, 9525, 14124, 4469, 8990, 2100, 9288, 64698, 10480,20893, 33230,1586, 9943, 16334, 68862, 9011, 14031, 6178, 21225, 1420, 32236, 2099, 2150, 26583, 2784, 8941, 9811, 27444, 50566 or 66428 molecules

PUBLICATION-DATE: August 21, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Hunter, John Joseph	Somerville	MA	US	
MacBeth, Kyle J.	Boston	MA	US	
Tsai, Fong-Ying	Newton	MA	US	
Lesoon, Andrea	Concord	MA	US	
Lightcap, Eric S.	Natick	MA	US	
Williamson, Mark J.	Saugus	MA	US	
Rudolph-Owen, Laura A.	Medford	MA	US	

APPL-NO: 10/ 354358

DATE FILED: January 30, 2003

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60353600 20020131 US

non-provisional-of-provisional 60364517 20020315 US

non-provisional-of-provisional 60371075 20020409 US

non-provisional-of-provisional 60371507 20020410 US

non-provisional-of-provisional 60372984 20020416 US

non-provisional-of-provisional 60374194 20020419 US

non-provisional-of-provisional 60382995 20020524 US

non-provisional-of-provisional 60385023 20020531 US

non-provisional-of-provisional 60388853 20020614 US

non-provisional-of-provisional 60389395 20020617 US

non-provisional-of-provisional 60391324 20020625 US

non-provisional-of-provisional 60395944 20020715 US

non-provisional-of-provisional 60397726 20020722 US

non-provisional-of-provisional 60403046 20020813 US

non-provisional-of-provisional 60405155 20020822 US

non-provisional-of-provisional 60406361 20020827 US

non-provisional-of-provisional 60421195 20021025 US

non-provisional-of-provisional 60425456 20021112 US

non-provisional-of-provisional 60427626 20021119 US

non-provisional-of-provisional 60432122 20021210 US

US-CL-CURRENT: 424/94.1, 435/6 , 514/44

ABSTRACT:

The present invention relates to methods for the diagnosis and treatment of a cancer or cancer. Specifically, the present invention identifies the differential expression of 140, 1470, 1686, 2089, 2427, 3702, 5891, 6428, 7181, 7660, 25641, 69583, 49863, 8897, 1682, 17667, 9235, 3703, 14171, 10359, 1660, 1450, 18894, 2088, 32427, 2160, 9252, 9389, 1642, 85269, 10297, 1584, 9525, 14124, 4469, 8990, 2100, 9288, 64698, 10480, 20893, 33230, 1586, 9943, 16334, 68862, 9011, 14031, 6178, 21225, 1420, 32236, 2099, 2150, 26583, 2784, 8941, 9811, 27444, 50566 and 66428 genes in tissues relating to cancer, relative to their expression in normal, or non-cancer disease states, and/or in response to manipulations relevant to a cancer. The present invention describes methods for the diagnostic evaluation and prognosis of various cancers, and for the identification of subjects exhibiting a predisposition to such conditions. The invention also provides methods for identifying a compound capable of modulating a cancer or cancer. The present invention also provides methods for the identification and therapeutic use of compounds as treatments of cancer.

RELATED APPLICATIONS

[0001] The present application claims the benefit of U.S. Provisional Application serial No. 60/353,600, filed on Jan. 31, 2002, of U.S. Provisional Application serial No. 60/364,517, filed on Mar. 15, 2002, of U.S. Provisional Application serial No. 60/371,075, filed on Apr. 9, 2002, of U.S. Provisional Application serial No. 60/371,507, filed on Apr. 10, 2002, of U.S. Provisional Application serial No. 60/372,984, filed on Apr. 16, 2002, of U.S. Provisional Application serial No. 60/374,194, filed on Apr. 19, 2002, of U.S. Provisional Application serial No. 60/382,995, filed on May 24, 2002, of U.S. Provisional Application serial No. 60/385,023, filed on May 31, 2002, of U.S. Provisional Application serial No. 60/388,853, filed on Jun. 14, 2002, of U.S. Provisional Application serial No. 60/389,395, filed on Jun. 17, 2002, of U.S. Provisional Application serial No. 60/391,324, filed on Jun. 25, 2002, of U.S. Provisional Application serial No. 60/395,944, filed on Jul. 15, 2002, of U.S. Provisional Application serial No. 60/397,726, filed on Jul. 22, 2002, of U.S. Provisional Application serial No. 60/403,046, filed on Aug. 13, 2002, of U.S. Provisional Application serial No. 60/405,155, filed on Aug. 22, 2002, of U.S. Provisional Application serial No. 60/406,361, filed on Aug. 27, 2002, of U.S. Provisional Application serial No. 60/421,195, filed on Oct. 25, 2002, of U.S. Provisional Application serial No. 60/425,456, filed on Nov. 12, 2002, of U.S. Provisional Application serial No. 60/427,626, filed on Nov. 19, 2002, and of U.S. Provisional Application serial No. 60/432,122, filed on Dec. 10, 2002.

The entire contents of these provisional patent applications are hereby incorporated by this reference.

---------- KWIC ---------

Summary of Invention Paragraph - BSTX (315):
[0314] The human 49863 sequence (SEQ ID NO:25), known also sphingosine kinase 1 (SPK-1), is approximately 1799 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acids 359 to 1513 of SEQ ID NO:25, encodes a 384 amino acid protein (SEQ ID NO:26).

Summary of Invention Paragraph - BSTX (419):
[0418] The human 64698 sequence (SEQ ID NO:77), known also as a sphingosine kinase 2 (SPK2), is approximately 2380 nucleotides long including untranslated regions. The coding sequence, located at about nucleic acids 7 to 1863 of SEQ ID NO:77, encodes a 618 amino acid protein (SEQ ID NO:78).

PGPUB-DOCUMENT-NUMBER: 20030125533

PGPUB-FILING-TYPE: new

DOCUMENT-IDENTIFIER: US 20030125533 A1

TITLE: Regulation of human sphingosine kinase-like protein

PUBLICATION-DATE: July 3, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Kossida, Sophia	Toulouse		FR	
Encinas, Jeffrey	Nara		JP	

APPL-NO: 09/ 969896

DATE FILED: October 4, 2001

RELATED-US-APPL-DATA:

non-provisional-of-provisional 60238005 20001006 US

non-provisional-of-provisional 60314113 20010823 US

US-CL-CURRENT: 536/23.1

ABSTRACT:

Reagents which regulate human sphingosine kinase-like protein activity and reagents which bind to human sphingosine kinase-like gene products can be used to regulate intracellular signaling and consequently cell proliferation and apoptosis. Such regulation is particularly useful for treating cancer, allergies including but not limited to asthma, autoimmune diseases such as rheumatoid arthritis, and central and peripheral nervous system disorders, such as Parkinson's disease.

[0001] This application claims priority to and incorporates by reference co-pending provisional applications Serial No. 60/238,005 filed Oct. 6, 2000 and 60/314,113 filed Aug. 23, 2001.

---------- KWIC ---------

Abstract Paragraph - ABTX (1):

Reagents which regulate human sphingosine kinase-like protein activity and reagents which bind to human sphingosine kinase-like gene products can be used to regulate intracellular signaling and consequently cell proliferation and apoptosis. Such regulation is particularly useful for treating cancer, allergies including but not limited to asthma, autoimmune diseases such as rheumatoid arthritis, and central and peripheral nervous system disorders, such as Parkinson's disease.

Summary of Invention Paragraph - BSTX (20):

[0018] Still another embodiment of the invention is a method of screening

for agents that can regulate an activity of a human sphingosine kinase-like protein, comprising the steps of contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of (a) the amino acid sequences shown in SEQ ID NOS: 2, 10, and 11 and (b) biologically active variants thereof; and detecting binding of the test compound to the polypeptide, wherein a test compound that binds to the polypeptide is identified as a potential agent for regulating the activity of the human sphingosine kinase-like protein.

Summary of Invention Paragraph - BSTX (21):

[0019] Yet another embodiment of the invention is a method of screening for therapeutic agents that can regulate an enzymatic activity of a human sphingosine kinase-like protein, comprising the steps of contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of (a) the amino acid sequences shown in SEQ ID NOS: 2, 10, and 11 and (b) biologically active variants thereof; and detecting the enzymatic activity of the polypeptide, wherein a test compound that increases the enzymatic activity of the polypeptide is identified as a potential therapeutic agent for increasing the enzymatic activity of the human sphingosine kinase-like protein, and wherein a test compound that decreases the enzymatic activity of the polypeptide is identified as a potential therapeutic agent for decreasing the enzymatic activity of the human sphingosine kinase-like protein.

Summary of Invention Paragraph - BSTX (22):

[0020] A further embodiment of the invention is a method of screening for therapeutic agents that can regulate an activity of a human sphingosine kinase-like protein, comprising the steps of contacting a test compound with a product encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of (a) the amino acid sequences shown in SEQ ID NOS: 2, 10, and 11 and (b) biologically active variants thereof; and detecting binding of the test compound to the product, wherein a test compound that binds to the product is identified as a potential therapeutic agent for regulating the activity of the human sphingosine kinase-like protein.

Summary of Invention Paragraph - BSTX (23):

[0021] Another embodiment of the invention is a method of reducing an activity of a human sphingosine kinase-like protein, comprising the step of contacting a cell comprising the human sphingosine kinase-like protein with a reagent that specifically binds to a product encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of (a) the amino acid sequences shown in SEQ ID NOS: 2, 10, and 11 and (b) biologically active variants thereof, whereby the activity of the human sphingosine kinase-like protein is reduced.

Summary of Invention Paragraph - BSTX (27):

[0025] A further embodiment of the invention is a method of treating a disorder selected from the group consisting of a cancer, an allergy, a CNS disorder, and an autoimmune disease, comprising the step of administering to a patient in need thereof a therapeutically effective dose of a reagent that inhibits a function of a human sphingosine kinase-like protein, wherein the human sphingosine kinase-like protein comprises an amino acid sequence selected from the group consisting of (a) the amino acid sequences shown in SEQ ID NOS: 2, 10, and 11 and (b) biologically active variants thereof, whereby symptoms of the disorder are ameliorated.

Brief Description of Drawings Paragraph - DRTX (3):
[0033] FIG. 2. Amino acid sequence of human sphingosine kinase-like protein (SEQ ID NO: 2). The diacylglycerol kinase catalytic domain is shown in bold.

Detail Description Paragraph - DETX (6):
[0040] Sphingosine kinase-like protein polypeptides according to the invention comprise an amino acid sequence as shown in SEQ ID NO: 2, a portion of SEQ ID NO: 2 comprising at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 320, or 326 contiguous amino acids, or a biologically active variant of the amino acid sequence shown in SEQ ID NO: 2, as defined below. Sphingosine kinase-like protein polypeptides according to the invention comprise an amino acid sequence as shown in SEQ ID NO: 10, a portion of SEQ ID NO: 10 comprising at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, or 537 contiguous amino acids, or a biologically active variant of the amino acid sequence shown in SEQ ID NO: 10, as defined below. Sphingosine kinase-like protein polypeptides according to the invention comprise an amino acid sequence as shown in SEQ ID NO: 11, a portion of SEQ ID NO: 11 comprising at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, or 562 contiguous amino acids, or a biologically active variant of the amino acid sequence shown in SEQ ID NO: 11, as defined below. A sphingosine kinase-like protein polypeptide of the invention therefore can be a portion of a sphingosine kinase-like protein molecule, a full-length sphingosine kinase-like protein molecule, or a fusion protein comprising all or a portion of a sphingosine kinase-like protein molecule.

Detail Description Paragraph - DETX (8):
[0042] Sphingosine kinase-like protein variants which are biologically active, i.e., retain a sphingosine kinase-like protein activity, also are sphingosine kinase-like protein polypeptides. Preferably, naturally or non-naturally occurring sphingosine kinase-like protein variants have amino acid sequences which are at least about 30, 35, 40, 45, 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to an amino acid sequence shown in SEQ ID NO: 2. Percent identity between a putative sphingosine kinase-like protein variant and an amino acid sequence of SEQ ID NO: 2 is determined using the Blast2 alignment program.

Detail Description Paragraph - DETX (10):
[0044] Amino acid insertions or deletions are changes to or within an amino acid sequence. They typically fall in the range of about 1 to 5 amino acids. Guidance in determining which amino acid residues can be substituted, inserted, or deleted without abolishing biological or immunological activity can be found using computer programs well known in the art, such as DNASTAR software. Whether an amino acid change results in a biologically active sphingosine kinase-like protein polypeptide can readily be determined by assaying for sphingosine kinase activity, as is known in the art and described, for example, in Liu, et al., J. Biol. Chem. 275, 19513-19520,2000.

Detail Description Paragraph - DETX (12):
[0046] Fusion proteins are useful for generating antibodies against sphingosine kinase-like protein amino acid sequences and for use in various assay systems. For example, fusion proteins can be used to identify proteins

which interact with portions of a sphingosine kinase-like protein polypeptide, including its active site and fibronectin domains. Methods such as protein affinity chromatography or library-based assays for protein-protein interactions, such as the yeast two-hybrid or phage display systems, can be used for this purpose. Such methods are well known in the art and also can be used as drug screens.

Detail Description Paragraph - DETX (13):

[0047] A sphingosine kinase-like protein fusion protein comprises two protein segments fused together by means of a peptide bond. Contiguous amino acids for use in a fusion protein can be selected from the amino acid sequence shown in SEQ ID NO: 2 or from a biologically active variants of those sequences, such as those described above. For example, the first protein segment can comprise at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, or 340 or more contiguous amino acids of SEQ ID NO: 2 or a biologically active variant. Sphingosine kinase-like protein polypeptides according to the invention comprise an amino acid sequence as shown in SEQ ID NO: 10, a portion of SEQ ID NO: 10 comprising at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, or 537 contiguous amino acids, or a biologically active variant of the amino acid sequence shown in SEQ ID NO: 10, as defined above. Sphingosine kinase-like protein polypeptides according to the invention comprise an amino acid sequence as shown in SEQ ID NO: 11, a portion of SEQ ID NO: 11 comprising at least 6, 10, 15, 25, 50, 75, 100, 125, 150, 175, 200, 225, 250, 275, 300, 325, 350, 375, 400, 425, 450, 475, 500, 525, 550, or 562 contiguous amino acids, or a biologically active variant of the amino acid sequence shown in SEQ ID NO: 11, as defined above. Preferably, a fusion protein comprises the active site of the protease and/or one or both of the fibronectin domains. The first protein segment also can comprise full-length sphingosine kinase-like protein.

Detail Description Paragraph - DETX (14):

[0048] The second protein segment can be a full-length protein or a protein fragment or polypeptide. Proteins commonly used in fusion protein construction include .beta.-galactosidase, .beta.-glucuronidase, green fluorescent protein (GFP), autofluorescent proteins, including blue fluorescent protein (BFP), glutathione-S-transferase (GST), luciferase, horseradish peroxidase (HRP), and chloramphenicol acetyltransferase (CAT). Additionally, epitope tags are used in fusion protein constructions, including histidine (His) tags, FLAG tags, influenza hemagglutinin (HA) tags, Myc tags, VSV-G tags, and thioredoxin (Trx) tags. Other fusion constructions can include maltose binding protein (MBP), S-tag, Lex a DNA binding domain (DBD) fusions, GAL4 DNA binding domain fusions, and herpes simplex virus (HSV) BP16 protein fusions. A fusion protein also can be engineered to contain a cleavage site located between the sphingosine kinase-like protein polypeptide-encoding sequence and the heterologous protein sequence, so that the sphingosine kinase-like protein polypeptide can be cleaved and purified away from the heterologous moiety.

Detail Description Paragraph - DETX (15):

[0049] A fusion protein can be synthesized chemically, as is known in the art. Preferably, a fusion protein is produced by covalently linking two protein segments or by standard procedures in the art of molecular biology. Recombinant DNA methods can be used to prepare fusion proteins, for example, by making a DNA construct which comprises sphingosine kinase-like protein coding sequences disclosed herein in proper reading frame with nucleotides encoding the second protein segment and expressing the DNA construct in a host cell, as is known in the art. Many kits for constructing fusion proteins are available

from companies such as Promega Corporation (Madison, Wis.), Stratagene (La Jolla, Calif.), CLONTECH (Mountain View, Calif.), Santa Cruz Biotechnology (Santa Cruz, Calif.), MBL International Corporation (MIC; Watertown, Mass.), and Quantum Biotechnologies (Montreal, Canada; 1-888-DNA-KITS).

Detail Description Paragraph - DETX (19):

[0053] A sphingosine kinase-like protein polynucleotide can be single- or double-stranded and comprises a coding sequence or the complement of a coding sequence for a sphingosine kinase-like protein polypeptide. A partial coding sequence of a sphingosine kinase-like protein polynucleotide is shown in SEQ ID NOS: 1 and 9.

Detail Description Paragraph - DETX (20):

[0054] Degenerate nucleotide sequences encoding human sphingosine kinase-like protein polypeptides, as well as homologous nucleotide sequences which are at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to the sphingosine kinase-like protein coding sequences nucleotide sequence shown in SEQ ID NO: 1 also are sphingosine kinase-like protein polynucleotides. Percent sequence identity between the sequences of two polynucleotides is determined using computer programs such as ALIGN which employ the FASTA algorithm, using an affine gap search with a gap open penalty of -12 and a gap extension penalty of -2. Complementary DNA (cDNA) molecules, species homologs, and variants of sphingosine kinase-like protein polynucleotides which encode biologically active sphingosine kinase-like protein polypeptides also are sphingosine kinase-like protein polynucleotides.

Detail Description Paragraph - DETX (22):

[0056] Variants and homologs of the sphingosine kinase-like protein polynucleotides disclosed above also are sphingosine kinase-like protein polynucleotides. Typically, homologous sphingosine kinase-like protein polynucleotide sequences can be identified by hybridization of candidate polynucleotides to known sphingosine kinase-like protein polynucleotides under stringent conditions, as is known in the art. For example, using the following wash conditions--2.times.SSC (0.3 M NaCl, 0.03 M sodium citrate, pH 7.0), 0.1% SDS, room temperature twice, 30 minutes each; then 2.times.SSC, 0.1% SDS, 50.degree. C. once, 30 minutes; then 2.times.SSC, room temperature twice, 10 minutes each--homologous sequences can be identified which contain at most about 25-30% basepair mismatches. More preferably, homologous nucleic acid strands contain 15-25% basepair mismatches, even more preferably 5-15% basepair mismatches.

Detail Description Paragraph - DETX (23):

[0057] Species homologs of the sphingosine kinase-like protein polynucleotides disclosed herein can be identified by making suitable probes or primers and screening cDNA expression libraries from other species, such as mice, monkeys, or yeast. Human variants of sphingosine kinase-like protein polynucleotides can be identified, for example, by screening human cDNA expression libraries. It is well known that the T.sub.m of a double- stranded DNA decreases by 1-1.5.degree. C. with every 1% decrease in homology (Bonner et al., J. Mol. Biol. 81, 123 (1973). Variants of human sphingosine kinase-like protein polynucleotides or sphingosine kinase-like protein polynucleotides of other species can therefore be identified, for example, by hybridizing a putative homologous sphingosine kinase-like protein polynucleotide with a polynucleotide having a nucleotide sequence of SEQ ID NOS: 1 and 9. The melting temperature of the test hybrid is compared with the

melting temperature of a hybrid comprising sphingosine kinase-like protein polynucleotides having perfectly complementary nucleotide sequences, and the number or percent of basepair mismatches within the test hybrid is calculated.

Detail Description Paragraph - DETX (24):

[0058] Nucleotide sequences which hybridize to sphingosine kinase-like protein polynucleotides or their complements following stringent hybridization and/or wash conditions are also sphingosine kinase-like protein polynucleotides. Stringent wash conditions are well known and understood in the art and are disclosed, for example, in Sambrook et al., MOLECULAR CLONING: A LABORATORY MANUAL, 2d ed., 1989, at pages 9.50-9.51.

Detail Description Paragraph - DETX (25):

[0059] Typically, for stringent hybridization conditions a combination of temperature and salt concentration should be chosen that is approximately 12-20.degree. C. below the calculated T.sub.m of the hybrid under study. The T.sub.m of a hybrid between a sphingosine kinase-like protein polynucleotide having a coding sequence disclosed herein and a polynucleotide sequence which is at least about 50, 55, 60, 65, 70, preferably about 75, 90, 96, or 98% identical to that nucleotide sequence can be calculated, for example, using the equation of Bolton and McCarthy, Proc. Natl. Acad. Sci. U.S.A. 48, 1390 (1962):

Detail Description Paragraph - DETX (30):

[0063] A naturally occurring sphingosine kinase-like protein polynucleotide can be isolated free of other cellular components such as membrane components, proteins, and lipids. Polynucleotides can be made by a cell and isolated using standard nucleic acid purification techniques, synthesized using an amplification technique, such as the polymerase chain reaction (PCR), or synthesized using an automatic synthesizer. Methods for isolating polynucleotides are routine and are known in the art. Any such technique for obtaining a polynucleotide can be used to obtain isolated sphingosine kinase-like protein polynucleotides. For example, restriction enzymes and probes can be used to isolate polynucleotide fragments which comprise sphingosine kinase-like protein nucleotide sequences. Isolated polynucleotides are in preparations which are free or at least 70, 80, or 90% free of other molecules.

Detail Description Paragraph - DETX (32):

[0065] Alternatively, synthetic chemistry techniques can be used to synthesize sphingosine kinase-like protein polynucleotides. The degeneracy of the genetic code allows alternate nucleotide sequences to be synthesized which will encode a sphingosine kinase-like protein polypeptide having, for example, the amino acid sequence shown in SEQ ID NOS: 2, 10, and 11 or a biologically active variant of that sequence.

Detail Description Paragraph - DETX (34):

[0067] Various PCR-based methods can be used to extend the nucleic acid sequences encoding the disclosed portions of human sphingosine kinase-like protein to detect upstream sequences such as promoters and regulatory elements. For example, restriction-site PCR uses universal primers to retrieve unknown sequence adjacent to a known locus (Sarkar, PCR Methods Applic. 2, 318-322, 1993). Genomic DNA is first amplified in the presence of a primer to a linker sequence and a primer specific to the known region. The amplified sequences

are then subjected to a second round of PCR with the same linker primer and another specific primer internal to the first one. Products of each round of PCR are transcribed with an appropriate RNA polymerase and sequenced using reverse transcriptase.

Detail Description Paragraph - DETX (45):

[0078] To express a sphingosine kinase-like protein polypeptide, a sphingosine kinase-like protein polynucleotide can be inserted into an expression vector which contains the necessary elements for the transcription and translation of the inserted coding sequence. Methods which are well known to those skilled in the art can be used to construct expression vectors containing sequences encoding sphingosine kinase-like protein polypeptides and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. Such techniques are described, for example, in Sambrook et al. (1989) and Ausubel et al., CURRENT PROTOCOLS IN MOLECULAR BIOLOGY, John Wiley & Sons, New York, N.Y., 1989.

Detail Description Paragraph - DETX (46):

[0079] A variety of expression vector/host systems can be utilized to contain and express sequences encoding a sphingosine kinase-like protein polypeptide. These include, but are not limited to, microorganisms, such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors, insect cell systems infected with virus expression vectors (e.g., baculovirus), plant cell systems transformed with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids), or animal cell systems.

Detail Description Paragraph - DETX (47):

[0080] The control elements or regulatory sequences are those non-translated regions of the vector--enhancers, promoters, 5' and 3' untranslated regions--which interact with host cellular proteins to carry out transcription and translation. Such elements can vary in their strength and specificity. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, can be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid lacZ promoter of the BLUESCRIPT phagemid (Stratagene, LaJolla, Calif.) or pSPORT1 plasmid (Life Technologies) and the like can be used. The baculovirus polyhedrin promoter can be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO, and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) can be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are preferable. If it is necessary to generate a cell line that contains multiple copies of a nucleotide sequence encoding a sphingosine kinase-like protein polypeptide, vectors based on SV40 or EBV can be used with an appropriate selectable marker.

Detail Description Paragraph - DETX (49):

[0082] In bacterial systems, a number of expression vectors can be selected depending upon the use intended for the sphingosine kinase-like protein polypeptide. For example, when a large quantity of a sphingosine kinase-like protein polypeptide is needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified

8>w can be used. Such vectors include, but are not limited to, multifunctional E. coli cloning and expression vectors such as BLUESCRIPT (Stratagene), in which the sequence encoding the sphingosine kinase-like protein polypeptide can be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of _-galactosidase so that a hybrid protein is produced. pIN vectors (Van Heeke & Schuster, J. Biol. Chem. 264, 5503-5509, 1989 or pGEX vectors (Promega, Madison, Wis.) can be used to express foreign Polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems can be designed to include heparin, thrombin, or Factor Xa protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

Detail Description Paragraph - DETX (52):

[0085] If plant expression vectors are used, the expression of sequences encoding sphingosine kinase-like protein polypeptides can be driven by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV can be used alone or in combination with the omega leader sequence from TMV (Takamatsu EMBO J. 6, 307-311, 1987). Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters can be used (Coruzzi et al., EMBO J. 3, 1671-1680, 1984; Broglie et al., Science 224, 838-843, 1984; Winter et al., Results Probl. Cell Differ. 17, 85-105, 1991). These constructs can be introduced into plant cells by direct DNA transformation or by pathogen-mediated transfection. Such techniques are described in a number of generally available reviews (see, for example, Hobbs or Murray, in MCGRAW HILL YEARBOOK OF SCIENCE AND TECHNOLOGY, McGraw Hill, New York, N.Y., pp. 191-196, 1992).

Detail Description Paragraph - DETX (53):

[0086] An insect system also can be used to express a sphingosine kinase-like protein polypeptide. For example, in one such system Autographa californica nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in Spodoptera frugiperda cells or in Trichoplusia larvae. Sequences encoding sphingosine kinase-like protein polypeptides can be cloned into a non-essential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of sphingosine kinase-like protein polypeptides will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses can then be used to infect, for example, S. frugiperda cells or Trichoplusia larvae in which sphingosine kinase-like protein polypeptides can be expressed (Engelhard et al., Proc. Nat. Acad. Sci. 91, 3224-3227, 1994).

Detail Description Paragraph - DETX (55):

[0088] A number of viral-based expression systems can be utilized in mammalian host cells. For example, if an adenovirus is used as an expression vector, sequences encoding sphingosine kinase-like protein polypeptides can be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome can be used to obtain a viable virus which is capable of expressing a sphingosine kinase-like protein polypeptide in infected host cells (Logan & Shenk, Proc. Natl. Acad. Sci. 81, 3655-3659, 1984). In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, can be used to increase expression in mammalian host cells.

Detail Description Paragraph - DETX (57):

[0090] Specific initiation signals also can be used to achieve more efficient translation of sequences encoding sphingosine kinase-like protein polypeptides. Such signals include the ATG initiation codon and adjacent sequences. In cases where sequences encoding a sphingosine kinase-like polypeptide, its initiation codon, and upstream sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals (including the ATG initiation codon) should be provided. The initiation codon should be in the correct reading frame to ensure translation of the entire insert. Exogenous translational elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression can be enhanced by the inclusion of enhancers which are appropriate for the particular cell system which is used (see Scharf et al., Results Probl. Cell Differ. 20, 125-162, 1994).

Detail Description Paragraph - DETX (59):

[0092] A host cell strain can be chosen for its ability to modulate the expression of the inserted sequences or to process an expressed sphingosine kinase-like protein polypeptide in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the polypeptidc also can be used to facilitate correct insertion, folding and/or function. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type Culture Collection (ATCC; 10801 University Boulevard, Manassas, Va. 20110-2209) and can be chosen to ensure the correct modification and processing of the foreign protein.

Detail Description Paragraph - DETX (60):

[0093] Stable expression is preferred for long-term, high-yield production of recombinant proteins. For example, cell lines which stably express sphingosine kinase-like protein polypeptides can be transformed using expression vectors which can contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the introduction of the vector, cells can be allowed to grow for 1-2 days in an enriched medium before they are switched to a selective medium. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sphingosine kinase-like protein sequences. Resistant clones of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Detail Description Paragraph - DETX (63):

[0096] Although the presence of marker gene expression suggests that the sphingosine kinase-like protein polynucleotide is also present, its presence and expression may need to be confirmed. For example, if a sequence encoding a sphingosine kinase-like protein polypeptide is inserted within a marker gene sequence, transformed cells containing sequences which encode a sphingosine kinase-like protein polypeptide can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding a sphingosine kinase-like protein polypeptide under the control of a single promoter. Expression of the marker gene in response to induction or

selection usually indicates expression of the sphingosine kinase-like protein polynucleotide.

Detail Description Paragraph - DETX (65):

[0098] The presence of a polynucleotide sequence encoding a sphingosine kinase-like protein polypeptide can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes or fragments or fragments of polynucleotides encoding a sphingosine kinase-like protein polypeptide. Nucleic acid amplification-based assays involve the use of oligonucleotides selected from sequences encoding a sphingosine kinase-like protein polypeptide to detect transformants which contain a sphingosine kinase-like protein polynucleotide.

Detail Description Paragraph - DETX (67):

[0100] A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding sphingosine kinase-like protein polypeptides include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, sequences encoding a sphingosine kinase-like protein polypeptide can be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and can be used to synthesize RNA probes in vitro by addition of labeled nucleotides and an appropriate RNA polymerase, such as T7, T3, or SP6. These procedures can be conducted using a variety of commercially available kits (Amershamn Pharmacia Biotech, Promega, and US Biochemical). Suitable reporter molecules or labels which can be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Detail Description Paragraph - DETX (69):

[0102] Host cells transformed with nucleotide sequences encoding a sphingosine kinase-like protein polypeptide can be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The polypeptide produced by a transformed cell can be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode sphingosine kinase-like protein polypeptides can be designed to contain signal sequences which direct secretion of sphingosine kinase-like protein polypeptides through a prokaryotic or eukaryotic cell membrane.

Detail Description Paragraph - DETX (70):

[0103] Other constructions can be used to join a sequence encoding a sphingosine kinase-like protein polypeptide to a nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp., Seattle, Wash.). The inclusion of cleavable linker sequences such as those specific for Factor Xa or enterokinase (Invitrogen, San Diego, Calif.) between the purification domain and the sphingosine kinase-like protein polypeptide can be used to facilitate

purification. One such expression vector provides for expression of a fusion protein containing a sphingosine kinase-like protein polypeptide and 6 histidine residues preceding a thioredoxin or an enterokinase cleavage site. The histidine residues facilitate purification on IMAC (immobilized metal ion affinity chromatography as described in Porath et al., Prot. Exp. Purif. 3, 263-281, 1992), while the enterokinase cleavage site provides a means for purifying the sphingosine kinase-like protein polypeptide from the fusion protein. Vectors which contain fusion proteins are disclosed in Kroll et al., DNA Cell Biol. 12, 441-453, 1993).

Detail Description Paragraph - DETX (72):

[0105] Sequences encoding a sphingosine kinase-like protein polypeptide can be synthesized, in whole or in part, using chemical methods well known in the art (see Caruthers et al., Nucl. Acids Res. Symp. Ser. 215-223, 1980; Horn et al. Nucl. Acids Res. Symp. Ser. 225-232, 1980). Alternatively, a sphingosine kinase-like protein polypeptide itself can be produced using chemical methods to synthesize its amino acid sequence. For example, sphingosine kinase-like protein polypeptides can be produced by direct peptide synthesis using solid-phase techniques (Merrifield, J. Am. Chem. Soc. 85, 2149-2154, 1963; Roberge et al., Science 269, 202-204, 1995). Protein synthesis can be performed using manual techniques or by automation. Automated synthesis can be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer). Various fragments of sphingosine kinase-like protein polypeptides can be separately synthesized and combined using chemical methods to produce a full-length molecule.

Detail Description Paragraph - DETX (73):

[0106] The newly synthesized peptide can be substantially purified by preparative high performance liquid chromatography (e.g., Creighton, PROTEINS: STRUCTURES AND MOLECULAR PRINCIPLES, WH Freeman and Co., New York, N.Y., 1983). The composition of a synthetic sphingosine kinase-like protein polypeptide can be confirmed by amino acid analysis or sequencing (e.g., the Edman degradation procedure; see Creighton, supra). Additionally, any portion of the amino acid sequence of the sphingosine kinase-like protein polypeptide can be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins to produce a variant polypeptide or a fusion protein.

Detail Description Paragraph - DETX (75):

[0108] As will be understood by those of skill in the art, it may be advantageous to produce sphingosine kinase-like protein polypeptide-encoding nucleotide sequences possessing non-naturally occurring codons. For example, codons preferred by a particular prokaryotic or eukaryotic host can be selected to increase the rate of protein expression or to produce an RNA transcript having desirable properties, such as a half-life which is longer than that of a transcript generated from the naturally occurring sequence.

Detail Description Paragraph - DETX (76):

[0109] The nucleotide sequences disclosed herein can be engineered using methods generally known in the art to alter sphingosine kinase-like protein polypeptide-encoding sequences for a variety of reasons, including modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides can be used to engineer the nucleotide sequences. For example, site-directed mutagenesis can be used to insert new restriction sites, alter glycosylation patterns, change codon preference, produce splice

variants, introduce mutations, and so forth.

Detail Description Paragraph - DETX (83):
[0116] In addition, techniques developed for the production of "chimeric antibodies," the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used (Morrison et al., Proc. Natl. Acad Sci. 81, 6851-6855, 1984; Neuberger et al., Nature 312, 604-608, 1984; Takeda et al., Nature 314, 452-454, 1985). Monoclonal and other antibodies also can be "humanized" to prevent a patient from mounting an immune response against the antibody when it is used therapeutically. Such antibodies may be sufficiently similar in sequence to human antibodies to be used directly in therapy or may require alteration of a few key residues. Sequence differences between rodent antibodies and human sequences can be minimized by replacing residues which differ from those in the human sequences by site directed mutagenesis of individual residues or by grating of entire complementarity determining regions. Alternatively, one can produce humanized antibodies using recombinant methods, as described in GB2188638B. Antibodies which specifically bind to a sphingosine kinase-like protein polypeptide can contain antigen binding sites which are either partially or fully humanized, as disclosed in U.S. Pat. No. 5,565,332.

Detail Description Paragraph - DETX (91):
[0124] Antisense oligonucleotides are nucleotide sequences which are complementary to a specific DNA or RNA sequence. Once introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form complexes and block either transcription or translation. Preferably, an antisense oligonucleotide is at least 11 nucleotides in length, but can be at least 12, 15, 20, 25, 30, 35, 40, 45, or 50 or more nucleotides long. Longer sequences also can be used. Antisense oligonucleotide molecules can be provided in a DNA construct and introduced into a cell as described above to decrease the level of sphingosine kinase-like protein gene products in the cell.

Detail Description Paragraph - DETX (93):
[0126] Modifications of sphingosine kinase-like protein gene expression can be obtained by designing antisense oligonucleotides which will form duplexes to the control, 5', or regulatory regions of the sphingosine kinase-like protein gene. Oligonucleotides derived from the transcription initiation site, e.g., between positions -10 and +10 from the start site, are preferred. Similarly, inhibition can be achieved using "triple helix" base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or chaperons. Therapeutic advances using triplex DNA have been described in the literature (e g., Gee et al., in Huber & Carr, MOLECULAR AND IMMUNOLOGIC APPROACHES, Futura Publishing Co., Mt. Kisco, N.Y., 1994). An antisense oligonucleotide also can be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Detail Description Paragraph - DETX (94):
[0127] Precise complementarity is not required for successful duplex formation between an antisense oligonucleotide and the complementary sequence of a sphingosine kinase-like protein polynucleotide. Antisense oligonucleotides which comprise, for example, 2, 3, 4, or 5 or more stretches of contiguous nucleotides which are precisely complementary to a sphingosine

kinase-like protein polynucleotide, each separated by a stretch of contiguous nucleotides which are not complementary to adjacent sphingosine kinase-like protein nucleotides, can provide targeting specificity for sphingosine kinase-like protein mRNA. Preferably, each stretch of complementary contiguous nucleotides is at least 4, 5, 6, 7, or 8 or more nucleotides in length. Non-complementary intervening sequences are preferably 1, 2, 3, or 4 nucleotides in length. One skilled in the art can easily use the calculated melting point of an antisense-sense pair to determine the degree of mismatching which will be tolerated between a particular antisense oligonucleotide and a particular sphingosine kinase-like protein polynucleotide sequence.

Detail Description Paragraph - DETX (98):

[0131] The coding sequence of a sphingosine kinase-like protein polynucleotide can be used to generate ribozymes which will specifically bind to mRNA transcribed from the sphingosine kinase-like protein polynucleotide. Methods of designing and constructing ribozymes which can cleave other RNA molecules in trans in a highly sequence specific manner have been developed and described in the art (see Haseloff et al. Nature 334, 585-591, 1988). For example, the cleavage activity of ribozymes can be targeted to specific RNAs by engineering a discrete "hybridization" region into the ribozyme. The hybridization region contains a sequence complementary to the target RNA and thus specifically hybridizes with the target (see, for example, Gerlach et al., EP 321,201).

Detail Description Paragraph - DETX (99):

[0132] Specific ribozyme cleavage sites within a sphingosine kinase-like protein RNA target are initially identified by scanning the RNA molecule for ribozyme cleavage sites which include the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides corresponding to the region of the sphingosine kinase-like protein target RNA containing the cleavage site can be evaluated for secondary structural features which may render the target inoperable. The suitability of candidate targets also can be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays. Longer complementary sequences can be used to increase the affinity of the hybridization sequence for the target. The hybridizing and cleavage regions of the ribozyme can be integrally related; thus, upon hybridizing to the sphingosine kinase-like protein target RNA through the complementary regions, the catalytic region of the ribozyme can cleave the target.

Detail Description Paragraph - DETX (101):

[0134] As taught in Haselof et al., U.S. Pat. No. 5,641,673, ribozymes can be engineered so that ribozyme expression will occur in response to factors which induce expression of a target gene. Ribozymes also can be engineered to provide an additional level of regulation, so that destruction of sphingosine kinase-like protein mRNA occurs only when both a ribozyme and a target gene are induced in the cells.

Detail Description Paragraph - DETX (103):

[0136] Described herein are methods for the identification of genes whose products interact with human sphingosine kinase-like protein. Such genes may represent genes which are differentially expressed in disorders including, but not limited to, cancer, allergies, CNS disorders, and autoimmune disease. Further, such genes may represent genes which are differentially regulated in response to manipulations relevant to the progression or treatment of such

diseases. Additionally, such genes may have a temporally modulated expression, increased or decreased at different stages of tissue or organism development. A differentially expressed gene may also have its expression modulated under control versus experimental conditions. In addition, the human sphingosine kinase-like gene or gene product may itself be tested for differential expression.

Detail Description Paragraph - DETX (107):

[0140] The differential expression information may itself suggest relevant methods for the treatment of disorders involving the human sphingosine kinase-like protein. For example, treatment may include a modulation of expression of the differentially expressed genes and/or the gene encoding the human sphingosine kinase-like protein. The differential expression information may indicate whether the expression or activity of the differentially expressed gene or gene product or the human sphingosine kinase-like gene or gene product are up-regulated or down-regulated.

Detail Description Paragraph - DETX (116):

[0149] Test compounds can be screened for the ability to bind to sphingosine kinase-like protein polypeptides or polynucleotides or to affect sphingosine kinase-like protein activity or sphingosine kinase-like protein gene expression using high throughput screening. Using high throughput screening, many discrete compounds can be tested in parallel so that large numbers of test compounds can be quickly screened. The most widely established techniques utilize 96-well microtiter plates. The wells of the microtiter plates typically require assay volumes that range from 50 to 500 .mu.l. In addition to the plates, many instruments, materials, pipettors, robotics, plate washers, and plate readers are commercially available to fit the 96-well format.

Detail Description Paragraph - DETX (126):

[0159] The two-hybrid system is based on the modular nature of most transcription factors, which consist of separable DNA-binding and activation domains. Briefly, the assay utilizes two different DNA constructs. For example, in one construct a polynucleotide encoding a sphingosine kinase-like protein polypeptide is fused to a polynucleotide encoding the DNA binding domain of a known transcription factor (eg., GAL-4). In the other construct, a DNA sequence that encodes an unidentified protein ("prey" or "sample") is fused to a polynucleotide that codes for the activation domain of the known transcription factor. If the "bait" and the "prey" proteins are able to interact in vivo to form an protein-dependent complex, the DNA-binding and activation domains of the transcription factor are brought into close proximity. This proximity allows transcription of a reporter gene (e.g., LacZ), which is operably linked to a transcriptional regulatory site responsive to the transcription factor. Expression of the reporter gene can be detected, and cell colonies containing the functional transcription factor can be isolated and used to obtain the DNA sequence encoding the protein which interacts with the sphingosine kinase-like protein polypeptide.

Detail Description Paragraph - DETX (135):

[0168] In another embodiment, test compounds which increase or decrease sphingosine kinase-like protein gene expression are identified. A sphingosine kinase-like protein polynucleotide is contacted with a test compound, and the expression of an RNA or polypeptide product of the sphingosine kinase-like protein polynucleotide is determined. The level of expression of sphingosine kinase-like protein mRNA or polypeptide in the presence of the test compound is

compared to the level of expression of sphingosine kinase-like protein mRNA or polypeptide in the absence of the test compound. The test compound can then be identified as a modulator of expression based on this comparison. For example, when expression of sphingosine kinase-like protein mRNA or polypeptide is greater in the presence of the test compound than in its absence, the test compound is identified as a stimulator or enhancer of sphingosine kinase-like protein mRNA or polypeptide is less expression. Alternatively, when expression of the mRNA or protein is less in the presence of the test compound than in its absence, the test compound is identified as an inhibitor of sphingosine kinase-like protein mRNA or polypeptide expression.

Detail Description Paragraph - DETX (149):

[0182] Cancer. Regulation of sphingosine kinase-like protein activity may provide a method of treating cancer. Cancer is a disease fundamentally caused by oncogenic cellular transformation. There are several hallmarks of transformed cells that distinguish them from their normal counterparts and underlie the pathophysiology of cancer. These include uncontrolled cellular proliferation, unresponsiveness to normal death-inducing signals (immortalization), increased cellular motility and invasiveness, increased ability to recruit blood supply through induction of new blood vessel formation (angiogenesis), genetic instability, and dysregulated gene expression. Various combinations of these aberrant physiologies, along with the acquisition of drug-resistance frequently lead to an intractable disease state in which organ failure and patient death ultimately ensue.

Detail Description Paragraph - DETX (164):

[0197] A reagent which affects sphingosine kinase-like protein activity can be administered to a human cell, either in vitro or in vivo, to reduce sphingosine kinase-like protein activity. The reagent preferably binds to an expression product of a human sphingosine kinase-like protein gene. If the expression product is a polypeptide, the reagent is preferably an antibody. For treatment of human cells ex vivo, an antibody can be added to a preparation of stem cells which have been removed from the body. The cells can then be replaced in the same or another human body, with or without clonal propagation, as is known in the art.

Detail Description Paragraph - DETX (186):

[0218] Purified sphingosine kinase-like protein polypeptides comprising a glutathione-S-transferase protein are absorbed onto glutathione-derivatized wells of 96-well microtiter plates are contacted with test compounds from a small molecule library at pH 7.0 in a physiological buffer solution. Sphingosine kinase-like protein polypeptides comprise the amino acid sequence shown in SEQ ID NOS: 2, 10, and 11. The test compounds comprise a fluorescent tag. The samples are incubated for 5 minutes to one hour. Control samples are incubated in the absence of a test compound.

Detail Description Paragraph - DETX (191):

[0222] RNA is isolated from the two cultures as described in Chirgwin el al., Biochem. 18, 5294-99, 1979). Northern blots are prepared using 20 to 30 .mu.g total RNA and hybridized with a .sup.32P-labeled sphingosine kinase-like protein-specific probe at 65.degree. C. in Express-hyb (CLONTECH). The probe comprises at least 11 contiguous nucleotides selected from the complement of SEQ ID NOS: 1 and 9. A test compound which decreases the sphingosine kinase-like protein-specific signal relative to the signal obtained in the absence of the test compound is identified as an inhibitor of sphingosine

kinase-like protein gene expression.

Detail Description Paragraph - DETX (193):
[0223] Treatment of a Tumor with a Reagent which Specifically Binds to a Sphingosine Kinase-Like Protein Gene Product

Detail Description Paragraph - DETX (194):
[0224] Synthesis of antisense sphingosine kinase-like protein oligonucleotides comprising at least 11 contiguous nucleotides selected from the complement of SEQ ID NOS: 1 and 9 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoroamidite procedure (Uhlmann et al., Chem. Rev. 90, 534-83, 1990). Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the Limulus Amebocyte Assay (Bang, Biol. Bull. (Woods Hole Mass.) 105, 361-362, 1953).

Detail Description Paragraph - DETX (197):
[0226] Treatment of a Rheumatoid Arthritis with a Reagent which Specifically Binds to a Sphingosine Kinase-Like Protein Gene Product

Detail Description Paragraph - DETX (198):
[0227] Synthesis of antisense sphingosine kinase-like protein oligonucleotides comprising at least 11 contiguous nucleotides selected from the complement of SEQ ID NOS: 1 and 9 is performed on a Pharmacia Gene Assembler series synthesizer using the phosphoroamidite procedure (Uhlmann et al., Chem. Rev. 90, 534-83, 1990). Following assembly and deprotection, oligonucleotides are ethanol-precipitated twice, dried, and suspended in phosphate-buffered saline (PBS) at the desired concentration. Purity of these oligonucleotides is tested by capillary gel electrophoreses and ion exchange HPLC. Endotoxin levels in the oligonucleotide preparation are determined using the Limulus Amebocyte Assay (Bang, Biol Bull. (Woods Hole, Mass.) 105,361-362, 1953).

Claims Text - CLTX (24):
24. A method of screening for agents that can regulate an activity of a human sphingosine kinase-like protein, comprising the steps of: contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of (a) the amino acid sequences shown in SEQ ID NOS: 2, 10, and 11 and (b) biologically active variants thereof; and detecting binding of the test compound to the polypeptide, wherein a test compound that binds to the polypeptide is identified as a potential agent for regulating the activity of the human sphingosine kinase-like protein.

Claims Text - CLTX (33):
33. A method of screening for therapeutic agents that can regulate an enzymatic activity of a human sphingosine kinase-like protein, comprising the steps of: contacting a test compound with a polypeptide comprising an amino acid sequence selected from the group consisting of (a) the amino acid sequences shown in SEQ ID NOS: 2, 10, and 11 and (b) biologically active variants thereof; and detecting the enzymatic activity of the polypeptide,

wherein a test compound that increases the enzymatic activity of the polypeptide is identified as a potential therapeutic agent for increasing the enzymatic activity of the human sphingosine kinase-like protein, and wherein a test compound that decreases the enzymatic activity of the polypeptide is identified as a potential therapeutic agent for decreasing the enzymatic activity of the human sphingosine kinase-like protein.

Claims Text - CLTX (38):

38. A method of screening for therapeutic agents that can regulate an activity of a human sphingosine kinase-like protein, comprising the steps of: contacting a test compound with a product encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of (a) the amino acid sequences shown in SEQ ID NOS: 2, 10, and 11 and (b) biologically active variants thereof; and detecting binding of the test compound to the product, wherein a test compound that binds to the product is identified as a potential therapeutic agent for regulating the activity of the human sphingosine kinase-like protein.

Claims Text - CLTX (41):

41. A method of reducing an activity of a human sphingosine kinase-like protein, comprising the step of: contacting a cell comprising the human sphingosine kinase-like protein with a reagent that specifically binds to a product encoded by a polynucleotide comprising a nucleotide sequence selected from the group consisting of (a) the amino acid sequences shown in SEQ ID NOS: 2, 10, and 11 and (b) biologically active variants thereof, whereby the activity of the human sphingosine kinase-like protein is reduced.

Claims Text - CLTX (57):

57. A method of treating a disorder selected from the group consisting of a cancer, an allergy, a CNS disorder, and an autoimmune disease, comprising the step of: administering to a patient in need thereof a therapeutically effective dose of a reagent that inhibits a function of a human sphingosine kinase-like protein, wherein the human sphingosine kinase-like protein comprises an amino acid sequence selected from the group consisting of (a) the amino acid sequences shown in SEQ ID NOS: 2, 10, and 11 and (b) biologically active variants thereof, whereby symptoms of the disorder are ameliorated.

US-PAT-NO: 6610534

DOCUMENT-IDENTIFIER: US 6610534 B2

TITLE: Induction of blood vessel formation through administration of polynucleotides encoding sphingosine kinases

DATE-ISSUED: August 26, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Liau; Gene	Darnestown	MD	N/A	N/A
Stefansson; Steingrimur	Gaithersburg	MD	N/A	N/A
Su; Joseph	Germantown	MD	N/A	N/A

APPL-NO: 09/ 970516

DATE FILED: October 4, 2001

PARENT-CASE:

This application claims the benefit under 35 USC .sctn.119(e) of the following United States provisional patent application: Provisional Application No. 60/238,230, filed on Oct. 5, 2000, for "Induction of Blood Vessel Formation Through Administration of Polynucleotides Encoding Sphingosine Kinases." The disclosure of that application is incorporated hereby by reference in its entirety.

US-CL-CURRENT: 435/320.1, 435/252.3 , 514/44 , 536/23.1 , 536/23.4 , 536/23.5

ABSTRACT:

A method of inducing blood vessel formation in an animal by administering to the animal a polynucleotide encoding a sphingosine kinase, or an analogue, fragment, or derivative thereof. The polynucleotide may be contained in an appropriate expression vector, such as a viral vector. The delivery of sphingosine kinase through administration of an expression vector which expresses sphingosine kinase provides for the formation of larger blood vessels containing a well defined structure that is supported by mural cells such as pericytes and smooth muscle cells.

12 Claims, 6 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 7

---------- KWIC ---------

Brief Summary Text - BSTX (3):

Vascular endothelial cells undergo morphogenesis into capillary networks in response to angiogenic factors. It was shown previously that sphingosine-1-phosphate, or SPP, a platelet-derived bioactive lipid, is an important sphingolipid-derived second messenger in mammalian cells that acts to

promote proliferation and to inhibit apoptosis. (Olivera, et al., Nature, Vol. 365, pgs. 557-560 (Oct. 7, 1993); Spiegel, et al., J. Leukoc. Biol., Vol. 65, No. 3, pgs. 341-344 (March 1999).) Recently, SPP was defined as a novel regulator of angiogenesis. (Lee, et al., Cell, Vol. 99, No. 3, pgs. 301-312 (Oct. 29, 1999).) SPP activates the endothelial cell differentiation genes (EDG) EDG-1 and EDG-3 subtypes of G protein-coupled receptors on endothelial cells. Both EDG-1 and EDG-3 regulated signaling pathways are required for endothelial cell morphogenesis into capillary-like networks. SPP induces the Gi/mitogen-activated protein kinase cell survival pathway and enhances small GTPase Rho and Rac coupled adherens junction assembly. (Lee, 1999.) The level of SPP is regulated potentially by the enzyme that catalyzes the phosporylation of sphingosine to SPP. The cloning and characterization of the first mammalian sphingosine kinases (murine SPHK1.alpha. and SPHK1.beta.) has been reported. (Kohama, et al., J. Biol. Chem., Vol. 273, No. 37, pgs. 23722-23728 (Sep. 11, 1998)). Human sphingosine kinases (SPHK1 and SPHK2) have also been reported. (Nava, et al., FEBS, 473:81-84 (2000) and Liu, et al., J. Biol. Chem., 275:19513-19520 (2000).)

Drawing Description Text - DRTX (4):
FIG. 2 shows the cDNA and amino acid sequences for murine sphingosine kinase 1.alpha..

Detailed Description Text - DETX (6):
In a preferred embodiment, the sphingosine kinase, or analogue, fragment, or derivative thereof is administered to the animal by administering to the animal an effective amount of a polynucleotide encoding a sphingosine kinase, or an analogue, fragment, or derivative thereof. The sphingosine kinase is mammalian, preferably primate, and most preferably human sphingosine kinase. Specific examples of sphingosine kinase amino acid sequences and the polynucleotides encoding them are found in Genbank for human SPHK1 and SPHK2 (accession numbers AF200328 and AF245447), mouse SPHK1.alpha., SPHK1.beta., and SPHK2 (accession numbers AF068748, AF068749, and AF245448), and rat SPHK1a, SPHK1c, SPHK1d, SPHK1e, and SPHK1f (accession numbers AB049571, AB049572, AB049573, AB049574, and AB049575). SEQ. ID NO:1 and SEQ ID NO:2 show the cDNA and amino acid sequences for human SPHK1. SEQ ID NO:3 and SEQ ID NO:4 show the cDNA and amino acid sequences for human SPHK2. An analogue of sphingosine kinase includes, but is not limited to, splice variants of sphingosine kinase, deletions in the coding region, and multiple forms (T. Kohama et al., JBC, 273:23722-23728 (1998); H. Liu et al., JBC, 275:19513-19520 (2000); Y. Banno, et al., Biochem J., 335:301-304 (1998)). A fragment of sphingosine kinase is a portion of the protein that retains its activity for inducing blood vessel formation. A derivative of sphingosine kinase includes, but is not limited to, modifications to alter sphingosine kinase regulation or biological activity. Non-limiting examples include the addition of a signal sequence to force secretion of the enzyme or modification of the calcium, calmodulin binding domain, ATP binding site, or membrane retention sequences. The polynucleotide is under the control of a suitable promoter. It is to be understood, however, that the scope of the present invention is not to be limited to any specific promoters.

Detailed Description Text - DETX (7):
Preferably, the polynucleotide encoding the sphingosine kinase, or an analogue, fragment, or derivative thereof is contained in an appropriate expression vehicle. Such expression vehicles include, but are not limited to, plasmids, eukaryotic vectors, prokaryotic vectors (such as, for example, bacterial vectors), and viral vectors. In one embodiment, the vector is a

viral vector. Viral vectors which may be employed include RNA virus vectors (such as retroviral vectors, including lentiviral vectors) and DNA virus vectors (such as adenoviral vectors, adeno-associated virus vectors, Herpes Virus vectors, and vaccinia virus vectors). When a DNA virus vector is employed in constructing the vector, the polynucleotide encoding the sphingosine kinase is in the form of DNA. When an RNA virus vector is employed in constructing the vector, the polynucleotide encoding the sphingosine kinase is in the form of RNA. Preferable viral vectors include adenoviral vectors (preferably lacking all viral genes, i.e. high capacity or gutless), lentiviral vectors (e.g. HIV, BIV-based), and adeno-associated virus (AAV) vectors.

Detailed Description Text - DETX (10):

In a preferred embodiment, the adenoviral vector comprises an adenoviral 5' ITR; an adenoviral 3' ITR; an adenoviral encapsidation signal; a DNA sequence encoding a sphingosine kinase, or an analogue, fragment, or derivative thereof, and a promoter controlling the DNA sequence encoding a sphingosine kinase, or an analogous, fragment, or derivative thereof. The vector is free of at least the majority of adenoviral E1 and E3 DNA sequences, but is not free of all of the E2 and E4 DNA sequences, and DNA sequences encoding adenoviral proteins promoted by the adenoviral major late promoter. In one embodiment, the vector also is free of at least a portion of at least one DNA sequence selected from the group consisting of the E2 and E4 DNA sequences.

Detailed Description Text - DETX (13):

Such a vector, in a preferred embodiment, is constructed first by constructing, according to standard techniques, a shuttle plasmid which contains, beginning at the 5' end, the "critical left end elements," which include an adenoviral 5' ITR, an adenoviral encapsidation signal, and an E1a enhancer sequence; a promoter (which may be an adenoviral promoter or a foreign promoter); a multiple cloning site (which may be as herein described); a poly A signal; and a DNA segment which corresponds to a segment of the adenoviral genome. The promoter may, in one embodiment, be a regulatable promoter, such as, for example, a glucocorticoid-responsive promoter or an estrogen-responsive promoter, or the promoter may be a tissue--specific promoter. The vector also may, in another embodiment, contain genomic elements which may increase and/or maintain expression of the DNA sequence encoding a sphingosine kinase, or an analogue, fragment, or derivative thereof. Such genomic elements include, but are not limited to, introns, exons, polyadenylation sequences, and 5' and 3' untranslated regions. Such genomic elements, and representative examples thereof, also are described in U.S. Pat. No. 5,935,935, issued Aug. 10, 1999. The vector also may contain a tripartite leader sequence. The DNA segment which corresponds to a segment of the adenoviral genome serves as a substrate for homologous recombination with an adenovirus. The plasmid may also include a selectable marker and an origin of replication. The origin of replication may be a bacterial origin of replication. Representative examples of such shuttle plasmids include pAvS6, which is described in published PCT Application Nos. WO 94/23582, published Oct. 27, 1994, and WO 95/09654, published Apr. 13, 1995, and in U.S. Pat. No. 5,543,328, issued Aug. 6, 1996. The DNA sequence encoding a sphingosine kinase, or an analogue, fragment, or derivative thereof then may be inserted into the multiple cloning site of the shuttle plasmid to produce a plasmid vector.

Detailed Description Text - DETX (16):

Through such homologous recombination, a vector is formed which includes an adenoviral 5' ITR, an adenoviral encapsidation signal; an E1a enhancer sequence; a promoter; a DNA sequence encoding a sphingosine kinase, or an

analogue, fragment, or derivative thereof; a poly A signal; adenoviral DNA sequences; and an adenoviral 3' ITR. The vector also may include a tripartite leader sequence. The vector may then be transfected into a helper cell line, such as the 293 helper cell line (ATCC No. CRL1573), which will include the E1a and the E1b DNA sequences, which are necessary for viral replication, and to generate adenoviral particles. Transfection may take place by electroporation, calcium phosphate precipitation, microinjection, or through proteoliposomes.

Detailed Description Text - DETX (24):

In another embodiment, the adenoviral vector is free of all adenoviral coding regions. This "gutless" adenoviral vector includes an adenoviral 5' ITR, an adenoviral packaging signal, a DNA sequence encoding sphingosine kinase or an analogue, fragment, or derivative thereof, and an adenoviral 3' ITR. The vector contains from about 26 kb to about 38 kb, preferably 28 kb to 32 kb, and may include one or more genomic elements.

Detailed Description Text - DETX (25):

The various adenoviral vectors may include promoters other than a sphingosine kinase promoter, such as tissue-specific promoters. The vector also may include, in addition to a DNA sequence encoding a sphingosine kinase, or an analogue, fragment, or derivative thereof, DNA sequences encoding additional proteins which facilitate the generation of new blood vessels, such as, but not limited to, vascular endothelial growth factors (VEGFs), fibroblast growth factors (FGFs), IGFs, angiopoietins, including angiopoietin 1, and angiopoietin 2, TGF-.beta., hypoxia inducible factors (HIFs) such as HIF1-.alpha., monocyte chemoattractant proteins (MCPs) such as MCP-1, nitric oxide synthase, ephrins, such as ephrin B2, and other angiogenic genes, platelet derived endothelial growth factor, and Interleukin-8.

Detailed Description Text - DETX (42):

The retroviral plasmid vector including the polynucleotide encoding a sphingosine kinase, or an analogue, fragment, or derivative thereof is transduced into a packaging cell line including nucleic acid sequences encoding the gag, pol, and env retroviral proteins. Examples of such packaging cell lines include, but are not limited to, the PE501, PA317 (ATCC No. CRL 9078), .PSI.-2, .PSI.-AM, PA12, T19-14X, VT-19-17-H2, .PSI.CRE, .PSI.CRIP, GP+E-86, GP+envAm12, and DAN cell lines as described in Miller, Human Gene Therapy, Vol. 1, pgs. 5-14 (1990), which is incorporated herein by reference in its entirety, or the 293T cell line (U.S. Pat. No, 5,952,225). The vector may transduce the packaging cells through any means known in the art. Such means include, but are not limited to, electroporation, the use of liposomes, and CaPO.sub.4 precipitation. Such producer cells generate infectious retroviral vector particles which include the polynucleotide encoding a sphingosine kinase, or an analogue, fragment, or derivative thereof.

Detailed Description Text - DETX (47):

Another embodiment has the expression of sphingosine kinase controlled by an inducible promoter. The use of an inducible gene expression system would allow the precise regulation of sphingosine kinase in a reversible manner. Several inducible systems are currently available. One example of a controlled promoter system in the Tet-On.TM. and Tet-Off.TM. systems currently available from Clontech (Palo Alto, Calif.). Tet-Off.TM. system uses the tetracycline-controlled transactivator (tTA), which is composed of the tet repressor protein (TetR) and the VP16 activation domain. tTA activates transcription in the absence of tetracycline. The Tet-On.TM. system uses the

reverse tetracycline-controlled transactivator (rtTA) and activates transcription in the presence of tetracycline. Both systems use the tetracycline-response element (TRE), which contains 7 repeats of the tet operator sequence, and the target gene, such as sphingosine kinase. tTA or rtTA bind to the TRE, activating transcription of the target gene. This promoter system allows the regulated expression of the transgene controlled by tetracycline or tetracycline derivatives, such as doxycycline. This system could be used to control the expression of sphingosine kinase in this instant invention.

Detailed Description Text - DETX (57):

The plasmid pCR3.1sphK1.alpha., derived from pCR3.1 (Invitrogen), was obtained from Thomas Baumruker (Novartis, Vienna, Austria) and contains the mouse sphingosine kinase alpha cDNA. pCR3.1sphK1x was digested with HindIII and NotI to isolate a 1,531 bp insert containing the coding sequence for sphK1.alpha.. The fragment was blunt-ended and cloned into the EcoRV site of pAVS6a1x, an adenoviral shuttle plasmid containing a lox recombination site, to create pAV1xsphK1.alpha. (FIG. 1). pAVS6a1x had been formed by adding a lox site to pAVS6a (U.S. Pat. No. 5,543,328). A 535 bp ClaI/NcoI fragment from pAVH8-1011x, containing the SV40 polyA signal and lox site was inserted into pAVS6a digested with ClaI and NcoI and linearized (4,745 bp). The sphK1.alpha. cDNA was cloned downstream of the RSV promoter and the adenoviral tripartite leader sequence, and included the SV40 polyadenylation signal and a homologous recombination region. A large-scale plasmid preparation was prepared using the alkaline lysis method and purified using a CsTFA gradient following standard protocols. The cDNA then was sequenced. The sphK1.alpha. coding sequence is 1,149 bp (SEQ ID NO:5) and encodes a 382 amino acid protein (SEQ ID NO:6). The cDNA and amino acid sequences are shown in FIG. 2.

Detailed Description Text - DETX (79):

Overexpression of Sphingosine Kinase protects cardiomyocytes from apoptosis: The AV3SK vector and its enzymatic product, sphingosine 1-phosphate (S-1-P) were both evaluated for possible inhibition of apoptosis in human cardiac myocytes induced by ceramide (n=3). heat shock (n=3), ischemia/reoxygenation (n=2). For vector-mediated studies, experiments were performed 3 days after vector treatment. The data, shown in FIG. 6, indicate that S-1-P is a potent inhibitor of human cardiac myocyte cell death. Av3SK transduced cells are also almost completely resistant to heat shock and ischemia/reoxygenation-induced apoptosis (FIG. 6). However, Av3SK vectors can only partially inhibit ceramide-induced apoptosis. This data in cardiac myocytes supports a cardioprotective role for sphingosine kinase and S1P. The use of a gene therapy vector to express sphingosine kinase represents a treatment modality for the long-term protection of cardiac myocytes from injury and protect against congestive heart failure.

US-PAT-NO: 6534323

DOCUMENT-IDENTIFIER: US 6534323 B1

TITLE: Compositions and methods for early detection of heart disease

DATE-ISSUED: March 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Sabbadini; Roger	Lakeside	CA	N/A	N/A

APPL-NO: 09/ 489466

DATE FILED: January 21, 2000

PARENT-CASE:

This application is related and claims priority to U.S. Provisional Application No. 60/049,274, filed on Jun. 10, 1997; and a Div. U.S. Application No. 09/084,069, filed on May 22, 1998, which issued as U.S. Pat. No. 6,210,976 B1 on Apr. 3, 2001.

US-CL-CURRENT: 436/518, 435/7.1 , 435/7.92 , 435/810 , 435/967 , 435/975 , 436/161 , 436/162 , 436/173 , 436/536 , 436/541 , 436/63 , 436/71 , 436/808 , 530/387.1 , 530/388.1 , 530/388.25 , 530/389.1 , 530/389.3 , 530/391.1 , 530/412 , 530/413 , 530/417

ABSTRACT:

The invention relates to methods, compositions, kits, and devices for detecting cardiac ischemia, hypoxia, or other causes of heart failure in a mammal by obtaining a test sample from a mammal, measuring a level of a non-polypeptidic cardiac marker in the test sample, and determining if the level of the cardiac marker measured in said test sample correlates with cardiac ischemia or hypoxia or another form of heart failure.

28 Claims, 1 Drawing figures

Exemplary Claim Number: 1

Number of Drawing Sheets: 1

---------- KWIC ---------

Detailed Description Text - DETX (116):

It has been shown that sphingosine inhibits PKC by preventing DAG binding to the enzyme (Faucher et al., J. Biol. Chem. 263:5319-5327, 1988). Thus, sphingosine may bind directly to PKC via the DAG binding site. The sequence for PKC.alpha. and its consensus DAG binding site is known (Hurley et al., Protein Science (6):477-80, 1997). Since SPH can also bind to putative sites on sphingosine kinase and other proteins with which is specifically interacts, it quite likely that several proteins have specific SPH binding sites. Accordingly, the putative sphingolipid binding site can be cloned using

standard techniques, after the screening of phage display libraries (see above) for colonies, which express the sphingolipid recognition site. Expression cloning of the cDNA of this protein would produce a reagent that could be used in a standard ELISA to detect sphingolipid changes in a blood sample.